# Autoimmunity

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# AUTOIMMUNITY

Sometimes structures of the own body are classified as foreign, resulting in an autoimmune reaction. Prevalence of autoimmune diseases in the general population is about 5% and rising, meaning approximately 385 million patients suffer from an autoimmune condition, globally. There are over 100 known autoimmune diseases, including: Hashimoto's thyroiditis, rheumatoid arthritis, type 1 diabetes, Sjögren's syndrome, Crohn's disease, Celiac disease, autoimmune hepatitis, vasculitis, and anti-phospholipid syndrome.

The trigger for autoimmunity remains unknown, although genetic predisposition and environmental factors such as stress, drugs or pollutants seem to play a role in their etiology. There may also be a cross-reactivity between antibodies against bacterial or viral antigens and self-antigens called molecular mimicry, where the foreign organisms have peptides similar to the self-antigens of the host. In this case, an immune response to a specific infection could also cause an autoimmune disease.

Most autoimmune diseases are associated with development of autoantibodies against structures in our own organs. These Antibodies can be measured by immunodiagnostic assays to determine specific disease, therapy efficacy or risk for disease development. This helps patients handle their disease, take preventive measures and seek personalized treatment, improving their quality of life.

The antigens in all our ELISAs are developed in-house using the latest technologies, and are produced recombinantly or purified from a native source to optimize clinical performance.

Our different kit configurations help improve your laboratory's flexibility while saving cost, whether you perform screening, profile, or single assays. In screening kits, different antigens are coated to one well for sensitive detection of positive patients, reducing the risk of missed disease. Positive patients can be tested with our profile kits to determine the specific type of disease, estimate the risk for disease development or the severity of the disease. In profile kits, each well of a row

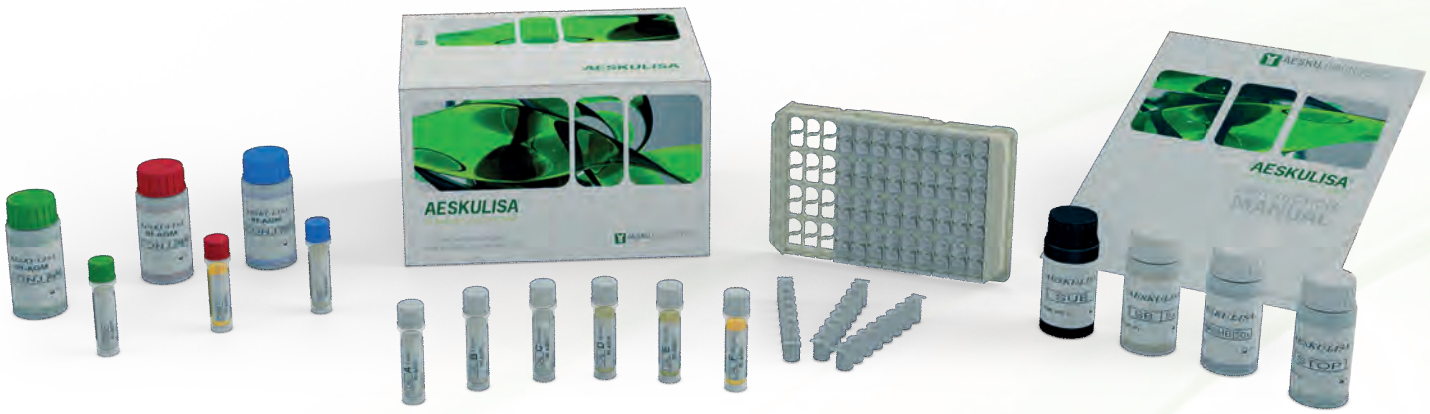
> *The **AESKULISA®** product line comprises the widest available range of standardized ELISA tests for all major autoimmune disease groups, from routine to research application.*

is coated with different antigens. End users may choose between quantitative or qualitative analysis. Highly-specific, single-parameter kits for quantitative determination or confirmation of qualitative results are also available.

# AESKULISA®

## SIMPLIFIES YOUR ELISA ROUTINE

# Kit Components

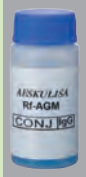



## Test principle

- Wells of the microtiter plate are coated with specific antigen.
- Serum sample from patients is added and during incubation specific antibody of positive patients binds to the antigen. Afterwards, unbound substances are washed away.
- In a second incubation step, specific antibodies are detected with an anti-human antibody that is linked to the enzyme horseradish peroxidase, (HRP). Afterwards, unbound antibody conjugates are washed away.
- HRP transforms the added substrate and a blue color appears. After addition of Stop Solution the reaction halts and color turns to yellow.
- Color intensity can be measured at a wavelength of 450 nm and is proportional to the antibody concentration in serum samples from patients.

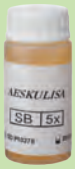
# ELISA TESTING

## Kit Configurations

| Antigen Coating 12 x 8 Wells MTP | | Conjugates | | | | Quantitative Results | Quali-tative Results | Quality-Controls |
|---|---|---|---|---|---|---|---|---|
| | |  IgG |  IgA |  IgM |  Mix |  6 x Calibrator 0–300 U/ml* |  Cut-off calibrator |  + Control - Control |
|  SCREENING | A | | ● | | | ● | ● | ● |
| | G | ● | | | | ○ | ● | ● |
| | GM | ● | | ● | | ○ | ● | ● |
|  PROFILE | A | | ● | | | | ● | ● |
| | G | ● | | | | ◖ | ● | ● |
| | GM | ● | | ● | | | ● | ● |
|  SINGLE | A | | ● | | | ● | ● | ● |
| | G | ● | | | | ● | ● | ● |
| | M | | | ● | | ● | ● | ● |
| | GM | ● | | ● | | ● | ● | ● |
| | GA | ● | ● | | | ● | ● | ● |
| | GAM | ● | ● | ● | | ● | ● | ● |
| | Check | | | | ● | ● | ● | ● |

+

**Common Reagents**




**Sample Buffer**


**Wash Buffer**


**TMB**


**Stop Solution**

○ Available for selected parameters
● Available for all parameters
◖ Semi quantitative available for some parameters or available for selected parameters with special calibration
* Calibration units may differ

# Rheumatology

Rheumatology deals with diseases of the human musculoskeletal system. Rheumatic disorders are chronic affecting joints and/or connective tissue. Approx. 14% of the general population suffers from pain in the musculoskeletal system and there are over 200 different conditions covered by the umbrella term rheumatic diseases. Major types are osteoarthritis, bursitis, tenosynovitis, capsulitis, fibromyalgia, neck or back pain and autoimmune rheumatic diseases.

Rheumatic diseases caused by autoimmunity include rheumatoid arthritis and connective tissue diseases such as systemic lupus erythematosus (SLE), Sjögren's syndrome, scleroderma, polymyositis/dermatomyositis (PM/DM) as well as mixed connective tissue disease (MCTD, Sharp syndrome). The prevalence of autoimmune rheumatic diseases is assumed to be between 1 and 2%.

The differentiation between these diseases is difficult, because their symptoms overlap and patients often have multiple autoimmune rheumatic diseases simultaneously, making diagnostic testing a key part of the clinical strategy.

The AESKU Rheumatology line offers a complete panel of routine and research usage kits, including more than 30 different parameters and over 40 kits.

All ***AESKULISA®*** antigens are also available in single tests for confirmation.

## Rheumatoid arthritis

Rheumatoid arthritis (RA) is the most common systemic autoimmune disease and approx. 1% of the general population suffers from RA. The disease occurs more often in women than in men. Mainly joints and associated structures are affected in RA, but other organs like skin, lung, kidney, liver and eyes can be involved.

Sometimes an overlap of RA and symptoms of connective tissue diseases can be found. This can change the appropriate therapy and therefore these diseases should be considered as well as clarified when there is suspicion of either disease.

## Diagnostics

Early and reliable diagnosis and prognosis of RA is mandatory for successful therapy since joint destruction can be significantly reduced or even prevented by appropriate drug intervention only in the early disease stages. Diagnosis of RA is based on 2010 ACR/EULAR classification criteria that evaluate joint involvement, duration of synovitis, acute phase reactants (erythrocyte sedimentation rate ESR, C-reactive protein CRP) and serology.

There are 3 main antibody types in patients with RA: rheumatoid factor (Rf), anti-cyclic citrullinated peptides antibodies (ACPA), and anti-nuclear antibodies (ANAs).

Rf are antibodies, directed against the Fc fragments of native human antibodies. IgM Rf antibodies can be detected before disease onset. Later, IgA and IgG antibodies also appear.

# RHEUMATOLOGY

CCP are post-translational citrullinated peptides from filaggrin, fibrin, fibrinogen, vimentin and type I as well as II collagens. Most of them are present intra – and extracellularly in the joints.

80% of RA patients are positive for Rf and 70–90% for anti CCP antibodies. In contrast to Rf, which is also found in other diseases such as Sjögren's syndrome. Antibodies to CCP are highly specific (95%) for RA. ANAs are found in only 41% of rheumatoid arthritis patients and the specificity is also low so they are tested for only in Rf or CCP negative cases where there is suspicion of RA.

These diagnostic assays are only useful for differential diagnosis of RA, since correlation of these markers to disease activity and progression is still under discussion.
Aggressive therapy is recommended only for those who are at risk of rapid joint destruction. Patients at low risk can be treated with corticoids, which have lower side effects than disease-modifying anti-rheumatic drugs (DMARD). There is a need for reliable markers that indicate disease activity and progression.

To meet this need, **AESKU.DIAGNOSTICS** developed the matrix metalloproteinase-3 (MMP-3) kit. MMP-3 is an enzyme that degrades different types of collagen, proteoglycans, fibronectin, laminin, and elastin. MMP-3 is directly involved in cartilage as well as bone degradation in RA.

MMP-3 levels increase during RA inflammation process and help predict bone erosion. In the early phase of the disease MMP-3 is also suitable for monitoring disease activity and therapy success, which is faster and cheaper than using activity scores like DAS, DAS28, SDAI or CDAI.

**Kits**
The ***AESKULISA®*** rheumatoid arthritis panel offers kits for screening and monitoring of diseases. Including Rf, ANAs and antibodies to CCP as well as synthetic citrullinated proteins/peptides (RA/CP-detect).
**AESKU.DIAGNOSTICS** also offers kits for simultaneous detection of IgA, IgG and IgM Rf antibodies, which allows cost-effective screening.


The ***AESKULISA®*** MMP-3 kit helps save valuable time and cost, since patients can receive the appropriate therapy in an early disease stage and can be monitored easily.

**References**

**1** Song Y.W., Kang E.H., Autoantibodies in rheumatoid arthritis: rheumatoid factors and anticitrullinated protein antibodies, QJM: An International Journal of Medicine, 2010;103(3):139-146, doi:10.1093/qjmed/hcp165

**2** Uemura Y., Hayashi H., Takahashi T., Saitho T., Umeda R., Ichise Y., Sendo S., Tsuji G., Kumagai S., MMP-3 as a Biomarker of Disease Activity of Rheumatoid Arthritis, Rinsho Byori, 2015;63(12):1357-6

## Systemic lupus erythematosus

In systemic lupus erythematosus (SLE) many different organs are affected by an immune response including the skin, blood, muscles, heart, lung or kidney making symptoms vary widely. Common symptoms are painful and swollen joints, fever, fatigue, chest pain, hair loss, swollen lymph nodes and rash.

SLE is the prototypic systemic autoimmune disease occurring in about 0.1% of the general population. SLE is more common in women, especially between 20 and 40 years, and may be linked to the hormone estrogen. Drug-induced lupus erythematosus (DILE) leads to similar symptoms as SLE.

### Diagnostics

A broad range of antibodies are associated with SLE. Anti-double stranded DNA (dsDNA) antibodies and anti-Smith antigen (Sm) antibodies are highly specific for the disease. However, other antibodies against histones, ribosomal-P proteins (Rib-P) or the ribonucleoprotein (RNP) also occur.

The former "American College of Rheumatology" (ACR) criteria have a sensitivity of 83% and a high specificity of 96%. Due to the relative low sensitivity some patients will not be recognized. Therefore, the new Systemic Lupus International Collaborating Clinics (SLICC) criteria was introduced, which has higher sensitivity (97%), but a lower specificity (84%).

A patient is classified as having SLE if he or she satisfies four of these criteria (at least one clinical and one immunological). A patient is also SLE positive if SLE suspected nephritis is proven by biopsy, and ANA or anti-dsDNA antibodies are detected.

Serological assays, therefore, play a critical role in diagnosis. Initially, an antinuclear antibody (ANA) screening by IFA and/or ELISA is recommended, since ANAs occur in 93% of SLE patients. However, ANAs also occur in 15% of healthy individuals and their prevalence increases with age. Due to that, ANA testing is suitable for screening, but more specific markers are also needed for final diagnosis.

Anti-dsDNA antibodies are rarely found in healthy individuals, but are in 30–40% of patients with general SLE. Almost all patients with renal problems show anti-dsDNA antibodies. They are also suitable for monitoring disease, since anti-dsDNA antibody concentration increases before disease flares up or at the latest with strong disease activity. In contrast, anti-single stranded DNA antibodies do not correlate with disease activity, but are often found in SLE patients with nephritis.

Like anti-DNA antibodies, anti-Sm antibodies are very specific for SLE. They occur in 20–30% of patients and often accompany kidney disease. 30–40% of patients with SLE are positive for anti-SS-A and/or anti-SS-B antibodies. However, these antibodies are more common in Sjögren's syndrome.

**References**

**1** Maidhof W, Hilas O. Lupus: An Overview of the Disease And Management Options. Pharmacy and Therapeutics. 2012;37(4):240-249

**2** Birtane M., Diagnostic Role of Anti-Nuclear Antibodies in Rheumatic Diseases, Archives of Rheumatology, 2012;27(2):079-089

**3** Petri M., Orbai A.-M., Alarcón G. S., et al. Derivation and Validation of Systemic Lupus International Collaborating Clinics Classification Criteria for Systemic Lupus Erythematosus, Arthritis and rheumatism, 2012;64(8):2677-2686, doi:10.1002/art.34473

# RHEUMATOLOGY

Histones are alkaline proteins, responsible for DNA packaging. DNA winds around histones resulting in nucleosome formation. Detection of anti-histone antibodies is important to distinguish between SLE and DILE, since 95% of DILE patients are positive for them but only 50–70% of those with SLE are positive. Moreover, DILE patients usually have ANAs, however, anti-ssDNA antibodies and anti-ENA antibodies are rarely detectable.

If SLE is suspected, but classical markers are missing, antibody tests for ribosomal-P proteins and nucleosome are recommended. Anti-nucleosome antibodies can be detected earlier than anti-dsDNA antibodies and 10% of patients with SLE have anti-rib-P antibodies, which are specific for this disease. Rib-P is a group of three phosphorylated proteins linked to the large subunit of ribosomes. Binding of antibodies, after internalization into the cell, can result in inhibition of protein synthesis.



**Kits**

***AESKULISA®*** DANA Pro allows a cost-saving differential diagnostic, if SLE is presumed. Antigens used are recombinant human dsDNA, U1-snRNP 70kDa, SS-B, Scl-70, Cenp-B, Jo-1, native human Sm and human recombinant 52 kDa SS-A/native 60 kDa SS-A. All antigens are also available in single kits.

In addition, the ***AESKULISA®*** SLE panel includes kits for detecting antibodies against different histones (C, H1, H2A, H2B, H3 and H4) and DNAs (IgA, IgG and IgM to ssDNA and dsDNA) as well as their complex (nucleosome). Moreover, IgG antibodies directed against ribosomal proteins P0, P1 and P2 can be recognized with our ***AESKULISA®*** Rib-P kit.

The ***AESKUSLIDES®*** nDNA *Crithidia luciliae* cells kits are suitable for reliable screening of patients with anti dsDNA antibodies.

The ***AESKULISA®*** phospholipid panel includes screening and profiling assays to measure up to eight different antigens. For screening in one well or profiling parallel in a row of eight wells, we offer the following antigens:

- Cardiolipin
- ß2-Glycoprotein
- Prothrombin
- Phosphatidylserine
- Phosphatidylethanolamine
- Phosphatidylinositol
- Phosphatidic acid
- Sphingomyelin

These tests are also available as single tests to confirm diagnosis results or to be used for therapy monitoring.

**Reference**

**1** Mok C.C., Lau C.S., Pathogenesis of systemic lupus erythematosus. Journal of Clinical Pathology, 2003;56(7):481-490

## Sjögren's syndrome

Sjögren's syndrome is an autoimmune disease of body's moisture-producing glands, specifically acinar and ductal cells. Symptoms that can include dry skin, vaginal dryness, a chronic cough, numbness in the arms and legs, feeling tired, muscle and joint pains, and thyroid problems.

## Diagnostics

Specific antigens in Sjögren's syndrome are SS-A, SS-B and alpha fodrin, in certain cases Rf will be detected. In 2012 the American College of Rheumatology (ACR) accepted the SICCA classification, which requires a positive test for at least two of the following three criteria:

- Positive serum anti-SS-A and/or anti-SS-B antibodies or positive rheumatoid factor and ANA ≥ 1:320
- Ocular staining score ≥ 3
- Presence of focal lymphocytic sialadenitis with focus score ≥ 1 focus/4 $mm^2$ in labial salivary gland biopsies

Testing for anti-SS-A and anti-SS-B antibodies (ab) shows a highly specific sensitivity of 84% and is 92% specific for Sjogren's syndrome (Sjs). Overlaps with other rheumatic disorders are feasible. In addition negative results for Rf ab exclude secondary Sjs.

In primary Sjs 93% of patients are positive for alpha fodrin antibodies, also 40% of RA patients and 20% of SLE patients show such ab. Additional to testing for anti-SS-A and anti-SS-B ab, testing against alpha-fodrin is recommended to evaluate for primary-Sjs.

## Kits

The ***AESKULISA®*** rheumatology panel offers single kits for confirmatory determination of antibodies against 52 kDa SS-A/60 kDa SS-A, SS-B and Rf.

The ***AESKULISA®*** alpha-Fodrin kits allows detection of anti-alpha fodrin IgA and/or IgG antibodies supporting a reliable diagnosis of primary Sjögren's syndrome after positive testing of anti-SS-A and anti-SS-B antibodies.


**References**

**1** Shiboski S.C., Shiboski C.H., Criswell L., et al. American College of Rheumatology classification criteria for Sjögren's syndrome: a data-driven, expert consensus approach in the Sjogren's International Collaborative Clinical Alliance cohort, Arthritis Care Res (Hoboken), 2012;64:475-487

**2** Ulbricht K. U., Schmidt R. E., Witte T., Antibodies against alpha-fodrin in Sjögren's syndrome, Autoimmunity Reviews, 2003;2(2):109-113

**3** Qin Q., Wang H., Wang H. Z., Huang Y. L., Li H., Zhang W. W., Zhang J. R., He L. L., Xia R., Zhao D. B., Deng A. M., Diagnostic accuracy of anti-alpha-fodrin antibodies for primary Sjögren's syndrome, Mod Rheumatol. 2014;24(5):793-7

# RHEUMATOLOGY

## Scleroderma

Scleroderma is a rare (0.1% of population affected) immune reaction to cells of the own skin. Distinctive symptoms are thickening, swelling as well as tightening of the skin and enlarged red blood vessels (telangiectasia). 90% of scleroderma patients show Raynaud's phenomenon where fingers are turning white and blue. Two main forms are described for scleroderma: limited and diffuse.
Limited scleroderma affects only the skin but shows a critical subtype called CREST syndrome. CREST stands for Calcinosis, Raynaud's phenomenon, Esophageal dysmotility (problem swallowing solid food), Sclerodactyly (thickening) and Telangiectasia.
The diffuse form of scleroderma also affects inner organs, such as hearth, intestinal system, kidneys and lung.

## Diagnostics

Major antibodies in Scleroderma are anti-Scl-70, anti-histone and anti-centromere protein antibodies. 90% of scleroderma (Scl) patients show auto-antibodies to nuclear antigens (ANAs). In IFA, the nucleolar ANA pattern for the diffuse scleroderma form is highly specific since it points to anti-PM-Scl antibodies. Moreover the centromere pattern indicates CREST syndrome. Anti-Cenp-B is highly specific (about 99%) for CREST syndrome but shows only 66% sensitivity in CREST syndrome patients. In diagnosis this high specificity is a big advantage since these antibodies can be detected years before onset of the disease.

Anti-Scl-70 can be found in 20–40% of patients with systemic sclerosis. Appearance of both Raynaud's phenomenon and anti-Scl-70 antibodies is highly specific for scleroderma (98%). In contrast, anti-PM-Scl antibodies point to polymyositis (PM) and scleroderma overlap.

It is therefore important to evaluate these parameters to guide the appropriate therapy. In unclear cases a skin biopsy is recommended in order to confirm diagnosis.

Anti-histone antibodies occur in 30% of patients with scleroderma. These antibodies are more common in SLE, however, they are also associated with pulmonary fibrosis in scleroderma.


## Kits

***AESKULISA®*** Sclero-Pro allows a cost-saving differential diagnosis of scleroderma. Antigens used are recombinant human PM-Scl, U1-snRNP 70kDa, SS-B, Scl-70, Cenp-B, Jo-1, native human Sm and human recombinant 52 kDa SS-A/native 60 kDa SS-A.

For confirmation of results we offer single kits for determination of antibodies against Scl-70, PM-Scl, histones and Cenp-B.

**References**

**1** Ho K.T., Reveille J.D., The clinical relevance of autoantibodies in scleroderma, Arthritis Research & Therapy, 2003;5(2):80-93

**2** Sato S., Ihn H., Kikuchi K., Takehara K., Anti-histone antibodies in systemic sclerosis: association with pulmonary fibrosis, Arthritis Rheum, 1994;37:391–4

## Polymyositis/Dermatomyositis

Autoimmune myositis represents a heterogeneous group of acquired muscle diseases. Their main clinical and morphological characteristics are muscle weakness, inflammation of skeletal muscles as well as Raynaud's phenomenon.

Important diseases in this group are polymyositis (PM) and dermatomyositis (DM), which have high morbidity and disability rates. Typical onset of PM or DM occurs between 31–53 years and the prevalence of each is approx. 0.01% of general population.

## Diagnostics

Serum levels of muscle-related enzymes are often increased in patients with PM or DM. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are also elevated.

Serological assays can clarify the situation, but negative ANA results do not exclude the disease, since only 60–80% of patients develop such antibodies. Nevertheless, ANA testing is useful for screening.

Anti-Jo-1 antibodies and anti-Mi-2 antibodies often are found in PM/DM patients and are highly specific for these diseases. While anti-Jo-1 antibodies are associated with PM, anti-Mi-2 antibodies suggest DM.

Antibodies to SS-A/Ro-52, Ku or PM/Scl are a sign of an overlapping syndrome. Particularly, anti-PM-Scl antibodies point to PM and scleroderma overlap. In contrast, high levels of anti-RNP antibodies indicate an overlap with mixed connective tissue disease. Presence of anti-SS-A/Ro-52 antibodies increases the risk for severe lung disease, so it is important to evaluate these parameters to choose the appropriate therapy.

## Kits

***AESKUBLOTS®*** Myositis Pro is designed to help in the diagnosis of poly- and dermatomyositis, as well as myositis-associated autoimmune diseases. Coated antigens are Jo-1, Mi-2, PM-Scl, U1-snRNP and Ku.

To confirm diagnosis results we offer single kits for determination of antibodies to U1-snRNP 70kDa, Jo-1, 52 kDa SS-A and 60 kDa SS-A.


**Reference**

**1** Cruellas M. G. P., dos Santos Trindade Viana V., Levy-Neto M., de Souza F. H. C., Shinjo S. K., Myositis-specific and myositis-associated autoantibody profiles and their clinical associations in a large series of patients with polymyositis and dermatomyositis, Clinics, 2013;68(7):909-914. doi:10.6061/clinics/2013(07)04

## Mixed connective tissue disease

Mixed connective tissue disease (MCTD/Sharp syndrome) combines features of scleroderma, myositis, SLE, and RA. The disease is very rare and major symptoms are joint pain and swelling, Raynaud's phenomenon, muscle inflammation, and thickening.

### Diagnostics

After positive ANA screening by IFA and/or ELISA, which occurs in 94% to 97% of MCTD patients, a profile assay for testing of antibodies to RNP, Sm, SS-A, SS-B, histones and dsDNA is recommended. Most of MCTD patients are positive for antibodies to RNP, but rarely for the other ANAs. In the absence of other ANAs, anti-RNP antibodies are a hint for MCTD.

Antibodies to Sm, SS-A, SS-B, histones, or dsDNA together with anti-RNP antibodies support the diagnosis of SLE. Anti-Jo-1 and anti-RNP antibodies indicate PM/DM. However, there is always the possibility of an overlapping syndrome.

| IFA ANA pattern | Presumable antigen | Presumable Disease |
|---|---|---|
| Speckled (fine) Speckled (coarse) | SS-A, SS-B, Scl-70, Jo-1, RNP, Sm, ribosomal-P proteins | SLE, MCTD, scleroderma, Sjögren's syndrome, polymyositis |
| Homogenous | dsDNA, histones, nucleosomes | SLE, drug-induced lupus (DIL) |
| Nucleolar | PM-Scl, RNP, Scl-70 | Scleroderma (SCL), polymyositis (PM) |
| Nuclear dots | Sp-100 | Primary biliary cirrhosis (PBC) |
| Centromere | Cenp A-E | Limited systemic sclerosis (CREST) |

However, the number of performed tests is rising, as the number of patients with MCTD increases, and clinical evaluation continues to be time consuming. Laboratories with heavy and complex workloads need to use methods with standardized results such as ELISA. This assay can be used as a first screening, but the ELISA has to be as sensitive as IFA. Moreover, it should have a reasonable specificity to exclude as many of ANA negative population as possible and avoid unnecessary and expensive follow-up testing.

**References**

**1** Cappelli S., Bellando Randone S., Martinovic D., et al. "To be or not to be," ten years after: evidence for mixed connective tissue disease as a distinct entity, Semin Arthritis Rheum, 2012;41:589-598

**2** Romatizmal, Hastalıklarda, Antinükleer, Antikorların, Tanısal, Rolü, Diagnostic Role of Anti-Nuclear Antibodies in Rheumatic Diseases, Turk J Rheumatol 2012;27(2):79-89

**3** Sacks J. J., Luo Y.-H., and Helmick C. G., Prevalence of Specific Types of Arthritis and Other Rheumatic Conditions in the Ambulatory Health Care System in the United States, 2001–2005, Arthritis Care & Research, 2010; 62(4):460–464, DOI 10.1002/acr.20041

**4** Solomon D. H., Kavanaugh A.J., Schur P.H., et al., Evidence-based guidelines for the use of immunologic tests: antinuclear antibody testing, Arthritis Rheum, 2002;47:434-444

To satisfy these needs, **AESKU.DIAGNOSTICS** developed a HEp-2 cell extract coated ELISA. Since assays without these extracts are significantly less sensitive and specific as relevant, not yet detected, antigens will be missing. The inclusion of spiked known antigens together with the nuclear HEp-2 cell extract significantly increases the sensitivity to detect ANAs.

After positive screening by IFA and/or ELISA to ANA a profile assay is recommended to identify the specific antibodies. To this end, immunoblots as well as profile ELISAs are very useful, which allow the differentiation of autoimmune rheumatic disease. These findings can be confirmed by single assays that are specific for the particular disease.

## Kits

***AESKULISA®*** ANA-8Pro allows cost-saving differential diagnosis. Antigens used are recombinant human U1-snRNP 70kDa, SS-B, Scl-70, Cenp-B, Jo-1, native human snRNP complex (snRNP/Sm), Sm and human recombinant 52 kDa SS-A/native 60 kDa SS-A. All antigens are also available as single kits.

***AESKUBLOTS®*** ANA-17 Pro is used for the differential diagnosis of systemic rheumatic diseases. Detection of autoantibodies in ***AESKUBLOTS®*** with the corresponding specific antigens allows an easier, cost-effective and more reliable differentiation of specific anti-nuclear antibodies (ANA's).

***AESKULISA®*** ANA HEp-2 is a screening-test using HEp-2 cell extracts spiked with recombinant antigens: SS-A-52, Cenp-B, SS-A-60, PM-Scl, SS-B, Jo-1, Sm and Scl-70. Allowing cost-effective and simultaneous detection of a variety of auto-antibodies in each well.

***AESKUSLIDES®*** ANA HEp-2 kits are suitable for reliable screening of patients with autoimmune rheumatic diseases.


**References**

**1** Colglazier C.L., Sutej P.G., Laboratory testing in the rheumatic diseases: a practical review, South Med J 2005;98:185-91

**2** Romatizmal, Hastalıklarda, Antinükleer, Antikorların, Tanısal, Rolü, Diagnostic Role of Anti-Nuclear Antibodies in Rheumatic Diseases, Turk J Rheumatol 2012;27(2):79-89

# RHEUMATOLOGY

## Spondyloarthritis

Axial spondyloarthritis (axSpA) is a chronic systemic rheumatoid disease, characterized by predominant involvement of the lumbar- and thoracic spine, and the sacroiliac joint. It appears in a low amount of patients with chronic back pain and shows a prevalence of 0.2–0.8%.

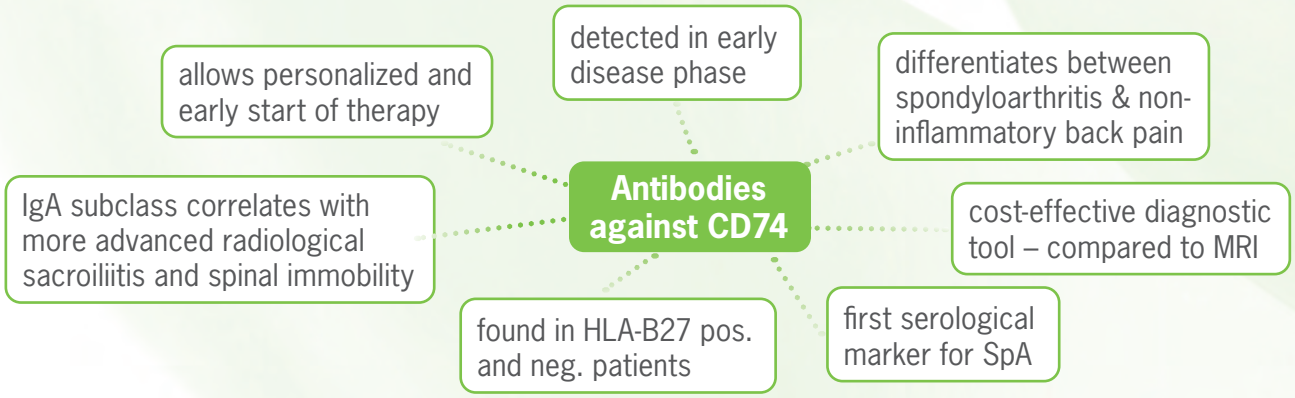
## Diagnostics

Diagnosis of axSpA is difficult, particularly in the early phase, since abnormalities in conventional X-Ray develop with a latency of several years. Currently, diagnosis of axSpA requires identification of sacroiliitis by medical imaging as part of the "Assessment of Spondyloarthritis international Society" (ASAS) criteria.

Magnetic resonance imaging (MRI) of the sacroiliac joints is considered the gold standard in diagnostics of SpA. MRI findings are the most sensitive (80%) and specific (more than 90%) for sacroiilitis.

Human leukocyte antigen (HLA)-B27 shows a good sensitivity, but is also present in up to 10% of healthy individuals. SpA is diagnosed by exclusion of other diseases with a considerable delay of 7–10 years between onset of inflammatory back pain (IBP) and axSpA diagnosis.

Recently, different studies successfully evaluated the role of antibodies to HLA class II-associated invariant chain peptide (anti-CD74) as an early and predictive diagnostic marker of SpA.





## Kit

***AESKULISA®*** SpA Detect is coated with recombinant human CD74 for quantitative and qualitative determination of IgA autoantibodies to CD74 in human sera. The test is intended to be used for SpA diagnosis (>90% sens./>90% spec.) and to clarify the origin of inflammatory or chronic back pain, and is not intended to be used for screening purposes nor the differential diagnosis of rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE).


**References**

**1** RKI Heft 53 Rückenschmerzen, 2012

**2** De Angelis R, Salaffi F, Grassi W. Prevalence of Spondyloarthropathies in an italien population sample: A regional community-based study. Scand J Rheumatol 2007;36:14–21

**3** Sieper J, Rudwaleit M, Baraliakos X, Brandt J, Braun J, Burgos-Vargas R, Dougados M, Hermann KG, Landewé R, Maksymowych W, van der Heijde D. The Assessment of SpondyloArthritis international Society (ASAS) handbook: a guide to assess spondyloarthritis. Ann Rheum Dis. 2009 Jun;68 Suppl 2:ii1-44

**4** Baerlecken N, Nothdorft S, Stummvoll G H, Sieper J, Rudwaleit M, Reuter S, Matthias T, Schmidt R E, Witte T. Autoantibodies against CD74 in spondyloarthritis. Ann Rheum Dis. 2013

# RHEUMATOLOGY *OVERVIEW*

| Ref No. | Product Name | Kit Configu-ration | Coated Antigen | Ig Class | Calibration/ Standard Range | Equivocal Zone/ Cut-off |
|---|---|---|---|---|---|---|
| *AUTOIMMUNITY* | | | | | | |
| *Rheumatology* | | | | | | |
| | ***AESKULISA®*** | | | | | |
| 3162 | alpha-Fodrin-A | Single | recombinant human α-fodrin | IgA | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3163 | alpha-Fodrin-G | Single | recombinant human α-fodrin | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3164 | alpha-Fodrin-Check | Single | recombinant human α-fodrin | IgAG | qualitative/quantitative (0–300 U/ml) | Equivocal: 16–24 U/ml Cut-off: 20 U/ml |
| 3100 | ANA-8S | Screen | mixture of 8 antigens recombinant: U1-snRNP 70kDa, SS-B, 52 kDa SS-A, Scl-70, Cenp-B, Jo-1 native: snRNP complex (snRNP/Sm), Sm, 60 kDa SS-A | IgG | qualitative | qualitative cut-off interpretation |
| 3101 | ANA-8Pro | Profile | 8 antigens separately recombinant: U1-snRNP 70kDa, SS-B, 52 kDa SS-A, Scl-70, Cenp-B, Jo-1 native: snRNP complex (snRNP/Sm), Sm, 60 kDa SS-A | IgG | qualitative | qualitative cut-off interpretation |
| 3115 | ANA HEp-2 | Single | lysed HEp-2 cells spiked with recombinant antigens | IgG | qualitative | qualitative cut-off interpretation |
| 3119 | ANA HEp-2 quantitative | Single | lysed HEp-2 cells spiked with recombinant antigens | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3102 | ENA-6S | Screen | mixture of 6 antigens recombinant: SS-B, 52 kDa SS-A, Scl-70, Jo-1 nativ: snRNP complex (snRNP/Sm), Sm, 60 kDa SS-A | IgG | qualitative | qualitative cut-off interpretation |
| 3103 | ENA-6Pro | Profile | 6 antigens separately recombinant: SS-B, 52 kDa SS-A, Scl-70, Jo-1 native: snRNP complex (snRNP/Sm), Sm, 60 kDa SS-A | IgG | qualitative/quantitative (0–100 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3116 | DANA-Pro | Profile | 8 antigens separately recombinant: dsDNA, U1-snRNP 70 kDa, SS-B, 52 kDa SS-A, Scl-70, Cenp-B, Jo-1; native: Sm, 60 kDa SS-A | IgG | qualitative | qualitative cut-off interpretation |
| 3121 | Sclero-Pro | Profile | 8 antigens separately recombinant: PM-Scl, U1-snRNP 70 kDA, SS-B, 52 kDa SS-A, Scl-70, Cenp-B, Jo-1 native: Sm, 60 kDa SS-A | IgG | qualitative | qualitative cut-off interpretation |

| Ref No. | Product Name | Kit Configu-ration | Coated Antigen | Ig Class | Calibration/ Standard Range | Equivocal Zone/ Cut-off |
|---|---|---|---|---|---|---|
| *AUTOIMMUNITY* | | | | | | |
| *Rheumatology* | | | | | | |
| | ***AESKULISA®*** | | | | | |
| 3104 | U1-70 | Single | recombinant human U1-snRNP 70 kDa | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal 12–18 U/ml Cut-off: 15 U/ml |
| 3105 | snRNP-C | Single | native human snRNP Complex (snRNP/Sm) | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal 12–18 U/ml Cut-off: 15 U/ml |
| 3106 | Sm | Single | native human Sm proteins | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3107 | SS-A | Single | recombinant human 52 kDa SS-A + native human 60 kDa SS-A | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3108 | SS-A-60 | Single | native human 60 kDa SS-A | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3109 | SS-A-52 | Single | recombinant human 52 kDa SS-A | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3110 | SS-B | Single | recombinant human SS-B | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3111 | Scl-70 | Single | recombinant human Scl-70 | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3112 | Cenp-B | Single | recombinant human 80 kDa Cenp-B | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3113 | Jo-1 | Single | recombinant human Jo-1 | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3114 | Rib-P | Single | native human ribosomal proteins P0, P1, P2 | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3117 | PM-Scl | Single | recombinant human PM-Scl | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3130 | Nucleo-h | Single | native human nucleosomes | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3141 | dsDNA-A | Single | recombinant human dsDNA | IgA | qualitative/quantitative (0–300 IU/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3142 | dsDNA-G | Single | recombinant human dsDNA | IgG | qualitative/quantitative (0–300 IU/ml) | Equivocal: 12–18 IU/ml Cut-off: 15 IU/ml |
| | | | International Standardization: NIBSC code 15/174 | | | |
| 3143 | dsDNA-M | Single | recombinant human dsDNA | IgM | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3140 | dsDNA-Check | Single | recombinant human dsDNA | IgA + IgG + IgM | qualitative/quantitative (0–300 U/ml) | Equivocal: 16–24 U/ml Cut-off: 20 U/ml |
| 3145 | ssDNA-A | Single | recombinant human ssDNA | IgA | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |

# RHEUMATOLOGY *OVERVIEW*

| Ref No. | Product Name | Kit Configu-ration | Coated Antigen | Ig Class | Calibration/ Standard Range | Equivocal Zone/ Cut-off |
|---|---|---|---|---|---|---|
| ***AUTOIMMUNITY*** | | | | | | |
| ***Rheumatology*** | | | | | | |
| | ***AESKULISA®*** | | | | | |
| 3146 | ssDNA-G | Single | recombinant human ssDNA | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3147 | ssDNA-M | Single | recombinant human ssDNA | IgM | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3144 | ssDNA-Check | Single | recombinant human ssDNA | IgA + IgG + IgM | qualitative/quantitative (0–300 U/ml) | Equivocal: 16–24 U/ml Cut-off: 20 U/ml |
| 3150 | Histone-C | Single | native human histone-complex | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3151 | Histone-H1 | Single | native human histone H1 | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3152 | Histone-H2A | Single | native human histone H2A | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3153 | Histone-H2B | Single | native human histone H2B | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3154 | Histone-H3 | Single | native human histone H3 | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3155 | Histone-H4 | Single | native human histone H4 | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3166 | CCP | Single | specific cyclic citrullinated peptides | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3161 | Rf-AGM | Single | Fc fragments of native human immunoglobulins IgG | IgA + IgG + IgM | qualitative/quantitative (0–300 U/ml) | IgA & IgG Equivocal: 12–18 U/ml Cut-off: 15 U/ml IgM Equivocal: 12–18 IU/ml Cut-off: 15 IU/ml |
| | | | International Standardization: IgM: NIBSC code W1066 | | | |
| 3160 | Rf-Check | Single | Fc fragments of native human immu-noglobulins IgG | IgA + IgG + IgM | qualitative/quantitative (0–300 U/ml) | Equivocal: 16–24 U/ml Cut-off: 20 U/ml |
| 3165 | RA/CP Detect | Single | synthetic cyclic citrullinated peptides | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3190 | SpA Detect | Single | recombinant human CD74 | IgA | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3168 | MMP-3 | Single | monoclonal antibodies against human MMP3 | | quantitative (0–200 ng/ml) | Equivocal: 20–30ng/ml women & 40–50ng/ml men Cut-off: >30 ng/ml women & >50 ng/ml men |

# Thyroid

There are two major autoimmune diseases that affect the thyroid. Hashimoto's thyroiditis is the most common and develops in 5–7% of the general population. Graves' disease is another type of autoimmune thyroid disorder with a prevalence of approx. 0.5%, and it is more common in the USA. Women are more likely to suffer from both diseases.

In Hashimoto's thyroiditis the immune response reduces the production of thyroid hormones leading to an underactive thyroid and higher levels of thyroid-stimulating hormones (TSH), resulting in symptoms such as fatigue, weight gain, feeling cold, joint and muscle pain, dry and thinning hair and depression. In Graves' disease the immune system often stimulates thyroid hormone production (overactive thyroid). This excess is reflected in irritability, muscle weakness, sleeping problems, tachycardia, diarrhea and subsequent weight loss.

It was shown in post-mortem studies that chronic autoimmune thyroiditis is present in 27% of adult women and 7% of adult men, which have not been diagnosed due to minor symptoms. The frequency of patients with higher levels of autoantibodies increases with age in both men and women.

**Diagnostics**

The most important autoantibodies are directed against thyroglobulin (Tg) and thyroperoxidase (TPO). In Graves' disease, antibodies against thyroid-stimulating hormone receptor (TSHR) are more common.

**Tg** is a protein iodinated by TPO, and afterwards cleaved by lysosomal proteases in up to 10 thyroid hormone molecules (T3 and T4 hormones).

**TPO** is an enzyme that oxidizes iodide ions and catalyzes the tyrosyl iodination of Tg. Anti-TPO antibodies are directed against the conformational epitopes of the carboxyl-terminal region of the TPO protein.

Often only thyroid-stimulating hormone (TSH) is measured in patients suspected to suffer from thyroid dysfunction. For accurate diagnosis of autoimmune thyroid dysfunction, autoantibodies against thyroglobulin (anti-Tg) and thyroperoxidase (anti-TPO) are very important markers, because either one or both appear in 98% of Hashimoto's thyroiditis patients.

Patients with Graves' disease also show anti-Tg (30%) and/or anti-TPO autoantibodies (70%). Since elevated thyroglobulin (Tg) serum levels are mainly not detected in patients with Hashimoto's thyroiditis (except occasionally in the beginning of disease), it can be measured to distinguish between other autoimmune diseases. Tg is also elevated in thyroid carcinoma and measurement can be useful in early detection as well as exclusion of metastases or tumor relapses.

Diagnostic tests help risk determination in autoimmune thyroid dysfunction since healthy individuals with low levels of anti-Tg antibodies are considered to be predisposed for this disease.

# THYROID


## Kits

All our thyroid kits are calibrated against international standards and feature excellent lot to lot consistency proved by continuous good scores in different international external quality control schemes for thyroid testing (UKNEQAS, DGKL, RCPA, CAP).

The ***AESKULISA***® Tg kit includes a recovery reaction to assure that the patient has no anti-Tg antibodies. The Tg recovery is performed by addition of a defined amount of Tg to exclude unspecific factors in determination of endogenous Tg. If the results do not show exactly the expected value, the patient has anti-Tg antibodies.

| Ref No. | Product Name | Kit Configuration | Coated Antigen | Ig Class | Calibration/ Standard Range | Equivocal Zone/ Cut-off |
|---|---|---|---|---|---|---|
| *AUTOIMMUNITY* | | | | | | |
| *Thyroid* | | | | | | |
| | ***AESKULISA***® | | | | | |
| 3400 | a-Tg | Single | native human thyroglobulin | IgG | qualitative/ quantitative (0–3000 IU/ml) | Equivocal: 120–180 IU/ml Cut-off: 150 IU/ml |
| | | | International Standardization: NIBSC code 65/093 | | | |
| 3401 | a-TPO | Single | human recombinant thyroid peroxidase | IgG | qualitative/ quantitative (0–3000 IU/ml) | Equivocal: 40–60 IU/ml Cut-off: 50 IU/ml |
| | | | International Standardization: NIBSC code 66/387 | | | |
| 3402 | Tg | | monoclonal anti-thyroglobulin antibodies | | quantitative (3.75-60 ng/ml) | |

**References**

**1** Vanderpump M. P. J., The epidemiology of thyroid disease, Br Med Bull, 2011; 99(1):39-51

**2** Iddah M.A. et al., Autoimmune Thyroid Disorders, ISRN Endocrinology, 2013; 2013:1-9

# Thrombosis

Thrombosis is a formation of blood clots inside a blood vessel. There are many causes leading to thrombosis including autoimmune conditions.

## Antiphospholipid syndrome

Antiphospholipid syndrome (APS) is an autoimmune disease characterized by antiphospholipid antibodies associated with thrombosis and/or pregnancy morbidity. These antibodies interfere with several factors of the coagulation system, producing a prothrombotic state.
APS may occur as an isolated disorder (primary APS) or in combination with other autoimmune diseases (secondary APS) such as systemic lupus erythematosus, rheumatoid arthritis and Sjögren's syndrome. Less than 1% of patients develop catastrophic APS associated with high mortality (50%).

## Diagnostics

Antiphospholipid antibodies are a heterogeneous group of autoantibodies directed against different anionic phospholipids and phospholipid binding proteins. ß2-glycoprotein I (ß2-GPI) appears to be the major binding protein, and cardiolipin the most important phospholipid antigen, both forming a complex. Approximately 90% of APS patients are anti-cardiolipin and/or anti-ß2-GPI antibody positive.
In accordance to the APS classification criteria, at least one clinical criterion (thrombotic event or gestational morbidity) and at least one laboratory criterion (positivity for anti-cardiolipin, anti-ß2-GPI or lupus anticoagulant) are required.
Furthermore, several "non-criteria" antiphospholipid antibodies against other anionic proteins or phospholipid-protein-complexes such as prothrombin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylglycerol, phosphatidic acid, sphingomyelin, laminin and annexin V can be found in patients. Testing for these antigenic targets can be important in patients with symptoms of APS but seronegative results for "classical" APS antibodies (10% of APS patients).

## Kits

**AESKU.DIAGNOSTICS** offers the most comprehensive panel of antiphospholipid Assays for separated as well as combined detection of antibodies:

- Highly purified cardiolipin
- Native human ß2-glycoprotein I of each IgG, IgM and/or IgA isotype
- Providing more flexibility and cost effective testing

Moreover, the ***AESKULISA***® phospholipid panel includes a wide range of antiphospholipid tests to increase both sensitivity and specificity of laboratory diagnosis.


**References**

**1** Cervera R et al. Euro-Phospholipid Project Group. Antiphospholipid syndrome: Clinical and immunologic manifestations and patterns of disease expression in a cohort of 1,000 patients. Arthritis Rheum, 2002;46:1019-1027

**2** Chaturvedi S, McCrae KR. Diagnosis and management of the antiphospholipid syndrome. Blood Rev, 2017;31(6):406-417

**3** Meroni PL et al. Pathogenesis of antiphospholipid syndrome: understanding the antibodies. Nat Rev Rheumatol, 2011;7(6):330-339

**4** Meroni PL et al. Antiphospholipid syndrome in 2014: more clinical manifestations, novel pathogenic players and emerging biomarkers. Arthritis Res Ther, 2014;16(2):209

**5** Miyakis S et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost, 2006;4(2):295-306

# THROMBOSIS

## Heparin-induced thrombocytopenia type II

Heparin-induced thrombocytopenia type II (HiT II) is a severe, immunological side effect in up to 5% of patients under anticoagulative treatment with heparin. In HiT II, administered heparin forms a molecular complex with platelet factor 4 (PF4), leading to the production of platelet-activating IgG autoantibodies. Because of that, thrombin release is increased while antithrombin-activating properties of heparin are limited.

Consequential serious complications are pulmonary embolism, skin necrosis and arterial occlusions leading to myocardial or cerebral infarction, amputation or death – mortality rate is up to 10%.

In the presence of HiT II, heparin administration has to be stopped immediately and substituted with alternative anticoagulants to prevent thrombosis. However, incorrect diagnosis of HiT II and an associated change in treatment in such patients could result in severe bleeding. Therefore, a method for rapid and exact diagnosis as well as efficient, standardized patient management is required.

### Diagnostics

Diagnosis of HiT II should be based on two pillars: First, determination of clinical parameters according to the so-called "4Ts Score" by experienced clinicians and second, serological analyses.
For rapid confirmation, immunoassays like ELISAs or immunoblots are suitable, which determine autoantibodies directed against a molecular complex of platelet factor 4 (PF4) and polyanionic heparin with high sensitivity. Patients with intermediate or high probability in the "4Ts Score" and positive serological values are considered to have HiT II.

For that reason reliable serological assays with high sensitivity and specificity for HiT II like:

- Platelet aggregation assay (PAA)
- Heparin-induced platelet activation assay (HIPA)
- Serotonin release assay (SRA)

are recommended subsequently.

Unfortunately, these analyses are time-consuming (up to days) and require special lab equipment, and cannot replace rapid immunoassays due to the need of fast decisions.


### Kits

Our ***AESKULISA***® HiT II panel includes sensitive as well as specific HiT II assays for detection of:

- IgA, IgG and IgM antibodies (HiT II Check)
- IgG antibodies only (HiT II)

against a molecular complex of platelet factor 4 (PF4) and polyanionic heparin.

**References**

**1** Arepally GM. Heparin-induced thrombocytopenia. Blood, 2017;129(21):2864-2872

**2** Cuker A et al. Predictive value of the 4Ts scoring system for heparin-induced thrombocytopenia: a systematic review and meta-analysis. Blood, 2012; 120(20):4160-4167

**3** Greinacher A et al. Close Approximation of Two Platelet Factor 4 Tetramers by Charge Neutralization Forms the Antigens Recognized by HIT Antibodies. Arteriosclerosis, Thrombosis, and Vascular Biology, 2006;26:2386-2393

**4** Smythe MA et al. The financial impact of heparin-induced thrombocytopenia, CHEST, 2008;134(3):568-573

**5** Smythe MA et al. Assessing the impact of a heparin-induced thrombocytopenia protocol on patient management, outcomes and cost. J Thromb Haemost, 2012;108:992-998

**6** Warkentin TE et al. scientific and standardization committee of the international society on thrombosis and hemostasis. Laboratory testing for heparin-induced thrombocytopenia: a conceptual framework and implications for diagnosis. J Thromb Haemost, 2011;9(12):2498-2500

| Ref No. | Product Name | Kit Configuration | Coated Antigen | Ig Class | Calibration/ Standard Range | Equivocal Zone/ Cut-off |
|---|---|---|---|---|---|---|
| *AUTOIMMUNITY* | | | | | | |
| *Thrombosis* | | | | | | |
| | ***AESKULISA®*** | | | | | |
| 3222 | Phospholipid-8Pro-A | Profile | 8 antigens separately native human: ß2-glycoprotein I, cardiolipin +ß2-glycoprotein I, cardiolipin, phosphatidylcholine, -ethanolamine, -inositol, -serine & sphingomyelin | IgA | qualitative | qualitative cut-off interpretation |
| 3232 | Phospholipid-8Pro-GM | Profile | 8 antigens separately native human: ß2-glycoprotein I, cardiolipin +ß2-glycoprotein I, cardiolipin, phosphatidylcholine, -ethanolamine, -inositol, -serine & sphingomyelin | IgG + IgM | qualitative | qualitative cut-off interpretation |
| 3216 | Phospholipid-Screen | Screen | each well contains native human ß2-glycoprotein I & cardiolipin, phosphatidylcholine,-ethanolamine, -inositol, -serine & sphingomyelin | IgG + IgM | qualitative | qualitative cut-off interpretation |
| 3219 | Phospholipid-Screen-A | Screen | each well contains native human ß2-glycoprotein I & cardiolipin, phosphatidylcholine, -ethanolamine, -inositol, -serine & sphingomyelin | IgA | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3224 | Phospholipid-Screen-GM | Screen | each well contains native human ß2-glycoprotein I & cardiolipin, phosphatidylcholine, -ethanolamine, -inositol, -serine & sphingomyelin | IgG + IgM | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3230 | Thrombo-Profile | Profile | separated antigens: native human ß2-glycoprotein I & cardiolipin, phosphatidylserine, -inositol & phosphatidic acid, as well the combination of the antigens +/- ß2-glycoprotein I | IgG + IgM | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3234 | APS-Profile-GM | Profile | 8 separated antigens: native human prothrombin, thrombin, cardiolipin, phosphatidylcholine, -ethanolamine, -inositol, -serine, sphingomyelin | IgG + IgM | qualitative | qualitative cut-off interpretation |
| 3203 | Cardiolipin-A | Single | native human cardiolipin + ß2-glycoprotein I<br><br>*calibrated against Harris sera* | IgA | qualitative/ quantitative (0–300 APL/ml) | Equivocal: 12–18 APL/ml Cut-off: 15 APL/ml |

# THROMBOSIS OVERVIEW

| Ref No. | Product Name | Kit Configuration | Coated Antigen | Ig Class | Calibration/ Standard Range | Equivocal Zone/ Cut-off |
|---|---|---|---|---|---|---|
| *AUTOIMMUNITY* | | | | | | |
| *Thrombosis* | | | | | | |
| | ***AESKULISA®*** | | | | | |
| 3204 | Cardiolipin-GM | Single | native human cardiolipin + ß2-glycoprotein I<br><br>*calibrated against Harris sera* | IgG + IgM | qualitative/ quantitative (0–300 GPL/ MPL/ml) | Equivocal: 12–18 GPL/MPL/ml Cut-off: 15 GPL/MPL/ml |
| 3244 | Cardiolipin-GM | Single | native human ß2-glycoprotein I + cardiolipin | IgG + IgM | qualitative/ quantitative (0–140 GPL/ MPL/ml) | Equivocal: 12–18 GPL/MPL/ml Cut-off: 15 GPL/MPL/ml |
| 3202 | Cardiolipin-Check | Single | native human cardiolipin + ß2-glycoprotein I<br><br>*calibrated against Harris sera* | IgA + IgG + IgM | qualitative/ quantitative (0–300 U/ml) | Equivocal: 16–24 U/ml Cut-off: 20 U/ml |
| 3205 | ß2-Glyco-A | Single | native human ß2-glycoprotein I | IgA | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3206 | ß2-Glyco-GM | Single | native human ß2-glycoprotein I | IgG + IgM | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3215 | ß2-Glyco-Check | Single | native human ß2-glycoprotein I | IgA + IgG + IgM | qualitative/ quantitative (0–300 U/ml) | Equivocal: 16–24 U/ml Cut-off: 20 U/ml |
| 3207 | Serine-GM | Single | native human ß2-glycoprotein I + phosphatidylserine | IgG + IgM | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3227 | Serine-Prothrombin-A | Single | native human prothrombin-phosphatidylserine-complex | IgA | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3226 | Serine-Prothrombin-GM | Single | native human prothrombin-phosphatidylserine-complex | IgG + IgM | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3223 | Serine-Prothrombin-Check | Single | native human prothrombin-phosphatidylserine-complex | IgG + IgM | qualitative/ quantitative (0–300 U/ml) | Equivocal: 16–24 U/ml Cut-off: 20 U/ml |

| Ref No. | Product Name | Kit Configuration | Coated Antigen | Ig Class | Calibration/ Standard Range | Equivocal Zone/ Cut-off |
|---|---|---|---|---|---|---|
| *AUTOIMMUNITY* | | | | | | |
| *Thrombosis* | | | | | | |
| | ***AESKULISA®*** | | | | | |
| 3210 | Prothrombin-A | Single | native human prothrombin | IgA | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3229 | Prothrombin-GM | Single | native human prothrombin | IgG + IgM | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3211 | Prothrombin-Check | Single | native human prothrombin | IgG + IgM | qualitative/ quantitative (0–300 U/ml) | Equivocal: 16–24 U/ml Cut-off: 20 U/ml |
| 3213 | Thrombin-A | Single | native human thrombin | IgA | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3228 | Thrombin-GM | Single | native human thrombin | IgG + IgM | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3225 | Thrombin-Check | Single | native human thrombin | IgA + IgG + IgM | qualitative/ quantitative (0–300 U/ml) | Equivocal: 16–24 U/ml Cut-off: 20 U/ml |
| 3208 | Inositol-GM | Single | native human ß2-glycoprotein I + phosphatidylinositol | IgG + IgM | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3236 | Ethanol-amine-A | Single | native human ß2-glycoprotein I + phosphatidylethanolamine | IgA | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3209 | Ethanol-amine-GM | Single | native human ß2-glycoprotein I + phosphatidylethanolamine | IgG + IgM | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3254 | Glycerol-A | Single | native human ß2-glycoprotein I + phosphatidylglycerol | IgA | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |

# THROMBOSIS OVERVIEW

| Ref No. | Product Name | Kit Configu-ration | Coated Antigen | Ig Class | Calibration/ Standard Range | Equivocal Zone/ Cut-off |
|---|---|---|---|---|---|---|
| **AUTOIMMUNITY** | | | | | | |
| *Thrombosis* | | | | | | |
| | ***AESKULISA®*** | | | | | |
| 3255 | Glycerol-GM | Single | native human ß2-glycoprotein I + phosphatidylglycerol | IgG + IgM | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3231 | Phosphatidic acid-GM | Single | native human ß2-glycoprotein I + phosphatidic acid | IgG + IgM | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3212 | Choline-GM | Single | native human ß2-glycoprotein I + phosphatidylcholine | IgG + IgM | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3214 | Sphingomy-elin-GM | Single | native human ß2-glycoprotein I + sphingomyelin | IgG + IgM | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3240 | Annexin V-GM | Single | native human annexin V | IgG + IgM | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3235 | Laminin | Single | native human laminin-1 | IgG | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3290 | HiT II | Single | HiT II-complex | IgG | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3291 | HiT II Check | Single | HiT II-complex | IgA + IgG + IgM | qualitative/ quantitative (0–300 U/ml) | Equivocal: 16–24 U/ml Cut-off: 20 U/ml |

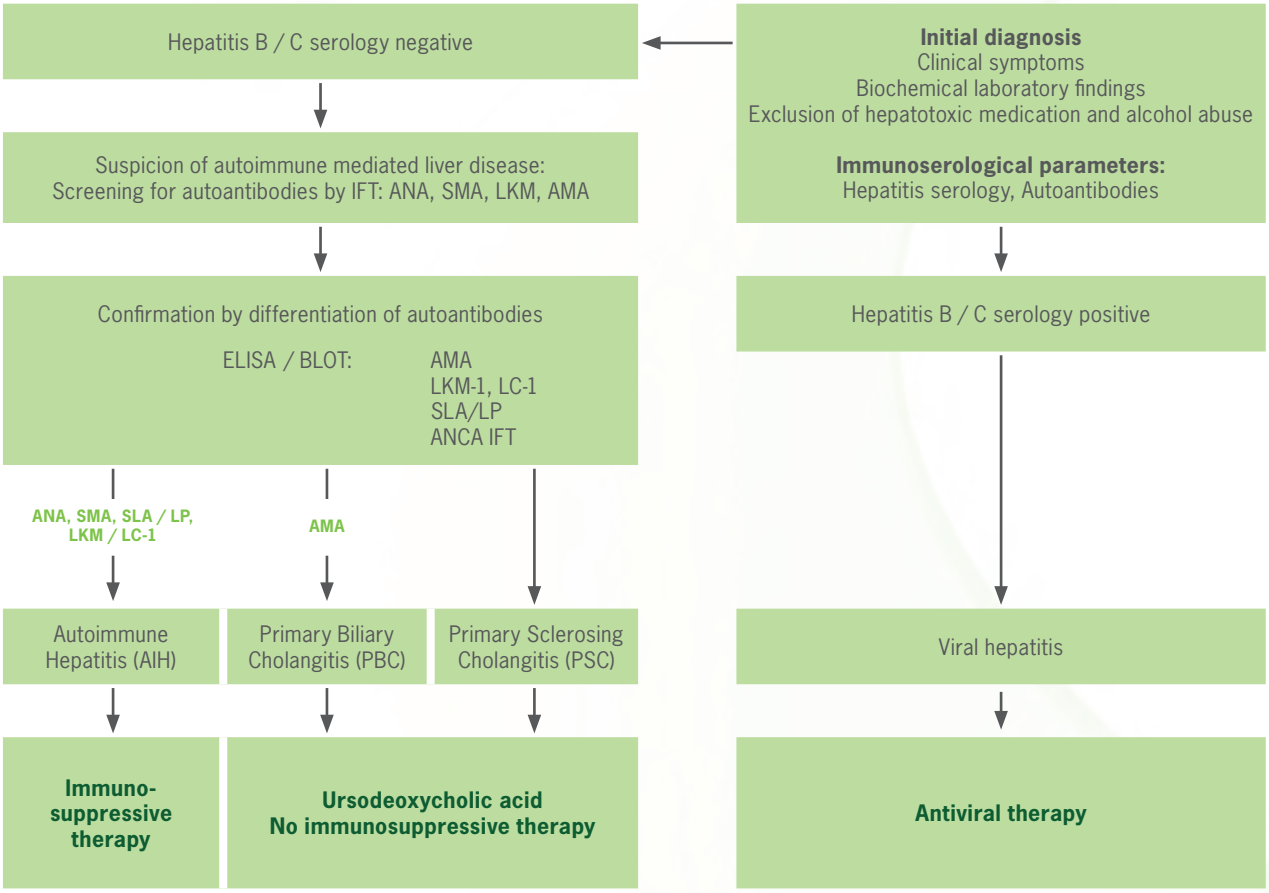
# Hepatology

Many kinds of liver diseases are known. While some are provoked by viruses like hepatitis A, B, C, D and E or G. Others are related to the immune system, drugs, poisons or alcohol. Yellowing of sclera and skin indicates liver disease. Further symptoms are weakness, fatigue and weight loss.

## Diseases

Liver diseases induced by autoimmunity include primary biliary cirrhosis (PBC), autoimmune hepatitis (AIH) and primary sclerosing cholangitis (PSC). The body's own immune cells destroy normal liver cells and normal tissue is replaced by connective tissue (fibrosis). When undetected, this can lead to liver cirrhosis, so an early and reliable diagnosis is greatly important.

## Example of a diagnosis algorithm for liver diseases




A definite clinical diagnosis should not be based on the results of the performed tests alone, but should be made by the physician after all clinical and laboratory findings have been evaluated. The diagnosis should be verified using different diagnostic methods.

**Reference**

Adapted and modified from an original algorithm from Czaja AJ and Norman GL. J Clin Gastroentero, 2003, 37: 315-329 by AESKU.DIAGNOSTICS.

**Autoantibodies/Antigens**

Due to the immune response, non-organ specific autoantibodies including anti-nuclear antibodies (ANA), anti-smooth muscle antibodies (ASMA), anti-mitochondrial antibodies (AMA) and antibodies to liver kidney microsome type-1 (LKM-1) are produced. Liver-specific autoantibodies such as antibodies to soluble liver antigen (SLA), liver cytosolic protein type 1 (LC-1) and asialoglycoprotein receptor (ASGPR) can also develop.

ANA concentration is elevated in many different diseases. They detect DNA and nuclear functional as well as structural proteins such as nuclear body antigen Sp100 and nuclear pore antigen gp210.

ASMAs recognize actin-containing filaments of muscle cells, troponin and tropomyosin.

Anti-LKM-1 antibodies are directed against P450 (CYP) 2D6, a cytochrome P450 mono-oxygenase that is found in the ribosomes of the endoplasmic reticulum of hepatocytes. They are also detectable in chronic viral hepatitis.

Anti-SLA/LP antibodies detect a soluble antigen that is identified as cytosolic UGA-suppressor transfer RNA-associated protein.

Anti-LC-1 antibodies bind to formiminotransferase cyclodeaminase (FTCD), which is a bifunctional enzyme with a transferase and deaminase activity. These antibodies are also present during chronic hepatitis C infections.

AMA-M2 recognizes the pyruvate dehydrogenase on the inner mitochondrial membrane. The most common AMA detects the E2 subunit of the dehydrogenase.

## Autoimmune hepatitis

In Autoimmune hepatitis (AIH), liver damage is caused by immune attack, so patients respond well to immunosuppressive therapy. AIH occurs in 0.015% of the population and affects more women than men. There are two groups of AIH. The classic form (type I) occurs more often (80%) and patients respond well to low dose steroids. AIH type II can be severe and is more common in female children.

## Diagnostic tests

Anti-nuclear antibodies (ANAs) and anti-smooth muscle antibodies (ASMAs) are important diagnostic markers for AIH type I. They are found in the sera of 52–85% of AIH patients and 22% of patients with primary biliary cirrhosis (PBC). A simultaneous occurrence of AIH and PBC or PSC is seen in approx. 10% or 6% of patients, respectively. In unclear cases, a liver biopsy is recommended to confirm diagnosis.

**AESKU.DIAGNOSTICS** is the patent co-owner of the recombinant soluble liver antigen/liver pancreas (SLA/LP). This antigen allows the detection of anti-SLA/LP antibodies, and is the only marker with specificity of 100% for AIH. However, it is not detectable by IFA and so use of ELISA is mandatory. SLA/LP antibodies are found in 10–50% of patients with AIH type I.

Antibodies against liver-kidney microsome type 1 (LKM-1) and liver cytosolic protein type 1 (LC-1) are predominantly found in AIH type II. High levels suggest a severe disease progression.

## Kits

***AESKULISA***®'s autoimmune liver disease panel offers a series of ELISAs including assays for

- anti-nuclear antibodies (ANA)
- anti liver/kidney microsomal (LKM) antibodies
- anti-soluble liver antigen/liver pancreas (SLA/LP) antibodies
- liver cytosolic protein type 1 (LC-1)

***AESKULISA***® ANA HEp-2 is a screening-test that is produced using HEp-2 cell extracts spiked with recombinant antigens:

- SS-A-52
- Cenp-B
- SS-A-60
- PM-Scl
- SS-B
- Jo-1
- Sm
- Scl-70


This allows cost effective and simultaneous detection of a variety of autoantibodies in one well.

***AESKUSLIDES***® rat and mouse liver/kidney/stomach slides allow the detection of antibodies against smooth muscles (ASMA) indicating AIH type I and antibodies against liver-kidney microsomes (LKM) indicating AIH type II.

***AESKUSLIDES***® ANA HEp-2 cells kits are suitable for reliable screening of anti-nuclear antibodies (ANAs) in patients with AIH.


**Reference**

**1** Wies I, Brunner S, Henninger J, Herkel J, Kanzler S, Meyer zum Büschenfelde KH, Lohse AW. Identification of target antigen for SLA/LP autoantibodies in autoimmune hepatitis. Lancet 2000; 355: 1510-1515

# HEPATOLOGY

## Primary biliary cirrhosis

Primary biliary cirrhosis (PBC) is an autoimmune disease in which intrahepatic bile ducts are affected. The prevalence of PBC is 0.04% and women are affected ten times more than men. Common symptoms are yellowing of the skin, itching and tiredness. Without appropriate treatment, it results in cirrhosis and eventually liver failure.

Previously, PBC was often diagnosed at the advanced stages of disease, where patients had already developed cirrhosis. Due to diagnostic blood tests, PBC can now be detected and addressed at earlier stages.

## Diagnostic tests

In PBC, anti-mitochondrial autoantibodies (AMA) are present in up to 95% of patients and in only 1% of the healthy population. Additionally, approx. 40% of AIH patients are positive for AMA, however, the antibody levels are much lower. For that reason, AMA are the most distinguishing serological parameter between AIH and PBC.

AMA are reliable predictive marker, because they are often detectable years before clinical symptoms appear. Since the IgM subclass is the first antibody class that arises in an immune response, it is important to test not only for IgG but also for IgM antibodies to reveal PBC at a very early stage.

5% of PBC patients are AMA negative and therefore it is also important to measure antibodies against nuclear body antigen Sp100 as well as nuclear pore antigen gp210. The prevalence of both is between 10% and 40% in PBC, however they are highly specific for PBC and these ANAs appear sometimes in the absence of AMAs. Moreover, anti-gp210 antibodies show an unfavorable prognosis.


## Kits

***AESKULISA***®'s autoimmune liver disease panel includes anti-mitochondrial IgG and IgM antibodies (AMA) ELISAs. **AESKU.DIAGNOSTICS** also offers an AMA test as a combined IgG and IgM antibody assay (***AESKULISA***® AMA-M2-Check).

***AESKUSLIDES***® rat and mouse liver/kidney/stomach slides also allow the detection of antibodies against mitochondria (AMA).

***AESKUBLOTS***® Liver Pro assay offers the possibility to detect the anti-nuclear antibodies (ANA) against gp210 and Sp100, which are highly specific for PBC, in addition to antibodies against:

- M2 (AMA)
- LKM-1
- LC-1
- SLA/LP

## Primary sclerosing cholangitis

Like PBC, Primary sclerosing cholangitis (PSC) affects intrahepatic bile ducts. However, in contrast to AIH and PBC, which are autoimmune diseases, PSC is a chronic inflammatory disease. The prevalence is below 0.01% and predominantly affects men (80%). Disease progression is often slow, however, left untreated it results in cirrhosis and liver failure.

80% of patients with PSC also have inflammatory bowel disease (IBD) – most often ulcerative colitis (UC) or sometime Crohn's disease. The biological connection is not known, but it is assumed immune cells are activated in the inflamed gut and migrate to the liver, resulting in inflammation.

### Diagnostic tests

In PSC patients almost no typical autoantibodies occur. Nevertheless, anti-neutrophil cytoplasmic antibodies (ANCA) are found in approx. 68% of patients with PSC and they are a hint for severe disease activity. ANCAs can also be detected in many AIH and some PBC patients. Both can be excluded by our

- anti-liver/kidney microsomal (LKM) antibody
- anti-soluble liver antigen/liver pancreas antigen (SLA/LP) antibody
- anti-liver cytosolic protein type 1 (LC-1) antibody
- anti-smooth muscles antibody (ASMA)
- anti-mitochondrial antibody (AMA) assays

since PSC patients are rarely positive for them. In unclear cases, a liver biopsy is recommended in order to confirm the diagnosis.


### Kits

***AESKUSLIDES®*** ANCA granulocytes slides allow detection of anti-neutrophil cytoplasmic antibodies (ANCA) by their specific ANCA pattern. Only perinuclear antigens of granulocytes (P-ANCA) is relevant in PSC.

**References**

**1** Himoto T., Nishioka M., Autoantibodies in liver disease: important clues for the diagnosis, disease activity and prognosis, Auto-Immunity Highlights, 2013;4(2):39-53, doi:10.1007/s13317-013-0046-7

**2** Tomizawa M., Shinozaki F., Fugo K., et al. Anti-mitochondrial M2 antibody-positive autoimmune hepatitis, Experimental and Therapeutic Medicine, 2015;10(4):1419-1422, doi:10.3892/etm.2015.2694

**3** Lindor K. D., Gershwin M. E., Poupon R., et al. "Primary Biliary Cirrhosis", Hepatology, 2009;50(1):291 308, doi:10.1002/hep.22906

**4** Hov J. R., Boberg K. M., Karlsen T. H., Autoantibodies in primary sclerosing cholangitis, World Journal of Gastroenterology : WJG, 2008;14(24):3781-3791, doi:10.3748/wjg.14.3781

# HEPATOLOGY *OVERVIEW*

| Ref No. | Product Name | Kit Configuration | Coated Antigen | Ig Class | Calibration/ Standard Range | Equivocal Zone/ Cut-off |
|---|---|---|---|---|---|---|
| *AUTOIMMUNITY* | | | | | | |
| *Hepatology* | | | | | | |
| | ***AESKULISA®*** | | | | | |
| 3705 | AMA-M2-G | Single | native mitochondrial M2-antigen | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3706 | AMA-M2-M | Single | native mitochondrial M2-antigen | IgM | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3707 | AMA-M2-Check | Single | native mitochondrial M2-antigen | IgG + IgM | qualitative/quantitative (0–300 U/ml) | Equivocal: 16–24 U/ml Cut-off: 20 U/ml |
| 3702 | LC-1 | Single | recombinant human formiminotransferase-cyclodeaminase | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3703 | LKM-1 | Single | recombinant human cytochrome p450 2D6 | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3704 | SLA/LP | Single | recombinant human-soluble liver-antigen/ liver-pancreatic-antigen (SLA/LP) | IgG | qualitative/quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |

# VASCULITIS

Vasculitis comprises a group of diseases that have an inflammation of the blood vessels in common. Arteries, capillaries and/or veins may be affected. Consequently, surrounding organs can be damaged due to insufficient blood supply. It occurs in 0.02% of the general population and the risk of developing the disease increases with age.

**Diseases**

The most common vasculitis is giant-cell arteritis or temporal arteritis, followed by small vessel vasculitides such as granulomatosis with polyangiitis (GPA, previously known as Wegener's granulomatosis), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA, previously known as Churg-Strauss syndrome). Together these diseases are termed antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAV).

Vasculitis occurs as a primary disease or as a consequence of another disease. Secondary vasculitis is often accompanied by rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), primary sclerosing cholangitis (PSC), cancer or hepatitis C infection.

In order to support the differential diagnosis of Goodpasture syndrome and/or GBM-disease, the relevant antibody detections are included here in the vasculitis panel.

**Diagnostics**

AAVs are associated with the presence of ANCAs. These antibodies are directed to cytoplasmic (C-ANCA) or perinuclear (P-ANCA) antigens of granulocytes. Specific patterns can be identified by immunofluorescence staining of granulocytes. The C-ANCA pattern predominantly indicates anti-proteinase 3 (PR3) antibodies. The P-ANCA pattern is caused by many different autoantibodies. It often points to antibodies against myeloperoxidase (MPO). Other targets such as lactoferrin, cathepsin G, elastase and lysozyme are also possible.
Atypical ANCA (A-ANCA) patterns generally do not conform to the P-ANCA and C-ANCA patterns. There is no distinct association to a specific antibody, but it is thought to be the same antigens as a P-ANCA pattern.
To differentiate vasculitis from Goodpasture-Syndrome or GBM disease, the detection of antibodies to the GBM is required. These antibodies often co-exist with anti-MPO or anti-PR3 antibodies.

## Kits

**AESKU.DIAGNOSTICS** offers you highly sensitive tools for detection and differentiation of ANCA with ***AESKUSLIDES***® ANCA Granulocyte kits and pattern recognition software (**HELPS+**** and **HELIOS**® software*).

For initial rapid and cost-effective screening of anti-neutrophil cytoplasmic antibodies (ANCAs), **AESKU.DIAGNOSTICS** offers our ***AESKULISA***® Vasculitis-Screen, which can detect anti-MPO and anti-PR3 antibodies simultaneously, and ***AESKUBLOTS***® Vasculitis Pro, which allows simultaneous detection and differentiation of anti-PR3, anti-MPO and anti-GBM antibodies.

Since there exists a variety of additional antigens like lactoferrin, cathepsin G, elastase, lysozyme and bactericidal/permeability increasing protein (BPI), the ***AESKULISA***® ANCA-Pro allows a rapid differentiation of these parameters.

To complete the portfolio, each marker can be quantified with its own specific ***AESKULISA***® kit. The ***AESKULISA***® PR3 sensitive assay is especially noteworthy because it offers higher sensitivity and specificity than the common direct ELISA setup, because of an innovative coating method exposing all relevant epitopes for binding of antibodies against proteinase 3.

***AESKULISA***® GBM helps identify patients with Goodpasture-Syndrome by detection of anti-GBM antibodies.

** Find out more in the brochure for AESKU.GROUP/ AUTOMATION.*


**References**

**1** Suresh E., Diagnostic approach to patients with suspected vasculitis, Postgrad Med J., 2006;82(970): 483 488

**2** Wagner A. et al. Autoantibodies in Systemic Vasculitis, Front Immunol., 2015;6:184

**3** Hagen E. C. et al. Diagnostic value of standardized assays for anti-neutrophil cytoplasmic antibodies in idiopathic systemic vasculitis. EC/ BCR Project for ANCA Assay Standardization, Kidney Int., 1998;53(3):743-53

**4** Olson S.W. et al. Asymptomatic autoantibodies associate with future anti-glomerular basement membrane disease. J Am Soc Nephrol. 2011 Oct. 22(10):1946-52

# VASCULITIS OVERVIEW

| Ref No. | Product Name | Kit Configuration | Coated Antigen | Ig Class | Calibration/ Standard Range | Equivocal Zone/ Cut-off |
|---|---|---|---|---|---|---|
| AUTOIMMUNITY | | | | | | |
| Vasculitis | | | | | | |
| | **AESKULISA®** | | | | | |
| 3323 | Vasculitis-Screen | Screen | mixture of 2 antigens native human: proteinase 3, myeloperoxidase | IgG | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3301 | ANCA-Pro | Profile | 7 antigens separately native human: proteinase 3, myeloperoxidase, neutrophilic elastase, cathepsin G, lactoferrin, lysozyme, BPI | IgG | qualitative/ quantitative (0–100 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3302 | PR3 sensitive | Single | native human proteinase 3 | IgG | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3303 | MPO | Single | native human myeloperoxidase | IgG | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3304 | BPI | Single | native human BPI | IgG | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3305 | Elastase | Single | native human neutrophilic elastase | IgG | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3306 | Cathepsin G | Single | native human cathepsin G | IgG | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3307 | Lactoferrin | Single | native human lactoferrin | IgG | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3308 | Lysozyme | Single | native human lysozyme | IgG | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3309 | GBM | Single | recombinant human GBM | IgG | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |

# Diabetes

Diabetes is a metabolic disease that impairs glucose uptake from the blood by cells. This is caused by a lack of insulin. Three main types of diabetes are known, type 1 diabetes (T1D), type 2 diabetes (T2D) and gestational diabetes (GDM).

## Disease

T1D is an autoimmune disease characterized by silent destruction of the β-cells of the pancreas. T1D occurs in about 10% of all cases of diabetes and most of these are diagnosed in people younger than 20.

Symptoms, such as frequent urination, thirst, weight loss, and poor wound healing, emerge when about 80–90% of the person's β-cells have been destroyed – years after the beginning of the destruction process. Insufficient/deficient insulin production results in hyperglycemia that can lead to life-threatening diabetic medical crisis.

## Diagnostics

T1D autoantibodies can be detected even if the patient is asymptomatic. Around 98% of new-onset T1D patients are positive for one or more autoantibodies. Clinical relevant antibodies are directed against glutamate decarboxylase 65 (GAD65), tyrosine phosphatase-related islet antigen 2 (IA2), insulin (IAA) and zinc transporter-8 (ZnT8).

Anti-Insulin antibodies are found in approximately 50% of children with new-onset T1D, but rarely in adulthood. Moreover, insulin antibodies are also produced after insulin therapy. Assays for the detection of IAA can not distinguish between antibodies against exogenous insulin and autoantibodies against endogenous insulin. Therefore, IAA testing of insulin-treated patients is not advised.

GAD65 is an enzyme predominantly found in the brain, but also in the pancreas. In 70–80% of new-onset T1D patients, GAD65 autoantibodies can be detected. Moreover, it is the most sensitive marker in patients having latent autoimmune diabetes in adults (LADA). These antibodies also occur in most patients with Stiff-Person-Syndrome, a neurological disease.

# DIABETES


Anti-IA2 autoantibodies are directed against the tyrosine phosphatase in the islet cell membrane and are specific for T1D. These autoantibodies occur in approximately 60% of new-onset diabetics. IA2 autoantibodies implicate a higher risk for the development of T1D, but they rarely appear alone.

Testing for T1D autoantibodies helps distinguish T1D from diabetes due to other causes. This can be important in a special form of adult-onset autoimmune diabetes – called LADA – that shares clinical and metabolic characteristics with both T1D and T2D. The presence of any islet cell autoantibody is one of the criteria to diagnose LADA.

As preclinical markers, T1D autoantibodies can be used to predict the risk of developing T1D, and that risk increases with each detectable antibody. For this reason, it is important to measure several T1D autoantibodies.

| Ref No. | Product Name | Kit Configuration | Coated Antigen | Ig Class | Calibration/ Standard Range | Equivocal Zone/ Cut-off |
|---|---|---|---|---|---|---|
| *AUTOIMMUNITY* | | | | | | |
| *Diabetes* | | | | | | |
| | ***AESKULISA®*** | | | | | |
| 3601 | Insulin | Single-G | recombinant human insulin | IgG | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12-18 U/ml Cut-off: 15 U/ml |

**References**

**1** Gillespie KM. Type 1 diabetes: pathogenesis and prevention. CMAJ, 2006;175(2):165-170

**2** Jacobsen LM, Haller MJ, Schatz DA. Understanding Pre-Type 1 Diabetes: The Key to Prevention. Front Endocrinol (Lausanne), 2018;9:70

**3** Pozzilli P, Pieralice S. Latent Autoimmune Diabetes in Adults: Current Status and New Horizons. Endocrinol Metab (Seoul), 2018;33(2):147-159

**4** Pietropaolo M, Towns R, Eisenbarth GS. Humoral Autoimmunity in Type 1 Diabetes: Prediction, Significance, and Detection of Distinct Disease Subtypes. Cold Spring Harbor Perspectives in Medicine, 2012;2(10):a012831

# Gastroenterology

Gastroenterology focuses on diseases of the digestive system, which includes stomach, intestine, liver, salivary glands, pancreas and gallbladder. Most common disorders are viral as well as bacterial infections, gallstones, tumors and chronic inflammatory diseases.

Classical symptoms are nausea, vomiting, diarrhea, bloating, constipation and/or fecal mucus. Continuous discomforts should be clarified, because they can be due to serious autoimmune diseases such as celiac disease, inflammatory bowel disease or pernicious anemia.

## Celiac disease

Celiac disease (CD) is a major public health issue, because it affects 1% of the population in certain regions and it is often underdiagnosed. Usually, the disease develops several years before it is properly diagnosed, and its causes are not fully understood. However, there is a genetic predisposition.

CD is caused by an immune reaction to gluten, which is found in wheat, barley and rye. The immune reaction affects the small intestine, but other organs can be involved due to the development of several different antibodies. Common symptoms are diarrhea, abdominal distension, malabsorption as well as anemia. Undetected, this disease increases the risk for lymphoma.

### Autoantibodies/Antigens

Gluten is a mixture of proteins. Most common disease inducing proteins are prolamins such as gliadin, which are rich in proline and glutamine. The enzyme tissue transglutaminase (tTg), found in the endomysium, can convert the glutamine in gliadin to glutamic acid. Resulting deamidated gliadin peptides (DGP) induce a stronger immune reaction. Tissue transglutaminase can also crosslink the glutamine in gliadin to lysine residues, forming a stable linked complex.

Due to mechanism of disease, most common antibodies are anti-gliadin antibodies (AGA), anti-tissue transglutaminase 2 antibodies (a-tTg), anti-deamidated gliadin peptide antibodies (a-DGP) and anti-tTg-gliadin-complex antibodies (tTg new generation).

## Diagnostic tests

Serological assays allow diagnosis of the disease before severe damage of the intestinal wall is visible by endoscopy. First diagnostic assays were developed for detection of anti-gliadin antibodies. However, the sensitivity (IgA: 73%/IgG: 73%) and the specificity (IgA: 90%/IgG: 80%) are fairly poor, since healthy individuals or patients with other diseases also have elevated serum levels. Anti-DGP antibody assays have since been developed, demonstrating higher sensitivity (84%) and specificity (90–99%). Sensitivity increases to 97%, if IgA and IgG antibodies are measured simultaneously.

# GASTROENTEROLOGY

Current standard for screening of CD is testing for anti-tTg IgA antibodies. Another reliable screening test is the detection of anti-endomysium antibodies (EMA) by IFA with primate esophagus as the antigen. Both show a sensitivity of approx. 95%. EMA testing is often performed to confirm anti-tTg IgA antibody screening, because of its higher specificity (almost 100% compared to 91%). Since 2–5% of CD patients show IgA antibody deficiency, IgG antibody tests need to be done additionally. Specifically, anti-DGP IgG antibody assays are recommended, having the highest specificity of all IgG antibody assays.

Antibodies to ately discovered neo epitope, directed against the tTg-gliadin-complex (tTg new generation) are proven to be more sensitive than the classical markers (tTg/EMA), because it is possible to detect formerly tTg seronegative CD patients. Moreover, anti-tTg new generation antibodies can be detected up to 15 months before anti-tTg or anti-DGP antibodies arise. Anti-tTg new generation antibodies are more suitable in prediction of diseases. In addition, they show a better correlation with mucosal damage (Marsh score) compared to classical markers.


**Kits**

**AESKU.DIAGNOSTICS** developed a unique marker for CD detection: The celiac neo-epitope used in our ***AESKULISA***® tTg New Generation kits. It can be used to screen adult as well as pediatric populations and IgA antibody deficient patients (Celicheck New Generation screens for IgA + IgG antibodies).

***AESKULISA***® CD panel also includes kits for detection of anti-alpha gliadin (Glia) IgA and/or IgG antibodies as well as anti-deamidated gliadin peptide (DGP) antibodies.

***AESKUSLIDES***® EMA primate esophagus slides allow detection of antibodies against endomysium (EMA), which includes antibodies against different tissue transglutaminases.

***AESKUBLOTS***® Gastro Pro assay is designed to assist in the diagnosis of CD, pernicious anemia and Crohn's disease. Celiac patients often have an IgA antibody deficiency. In order to avoid false-negative results, ***AESKUBLOTS***® Gastro Pro detects IgA and IgG antibodies. Antigens coated are gliadin, neo-tTg, mannan (ASCA), parietal cell-antigen (PCA) and intrinsic factor.

## Inflammatory bowel disease

Inflammatory bowel disease (IBD) is categorized into two main forms: Crohn's disease and ulcerative colitis (UC). Both are chronic inflammatory disorders that affect the intestine. Usually the diseases are detected in patients between 20 and 40 years or in patients over 60 years. Prevalence is approx. 0.01% and 0.007% for UC and Crohn's disease, respectively.

In both, a genetic predisposition is present. It is assumed that pathogens such as listeria, mycobacteria, as well as *Saccharomyces cerevisiae* can trigger these diseases, but this assumption lacks strong experimental support. Importantly, the diversity of microbiota and the count of bacteria are reduced in Crohn's disease and UC. For that reason, the intestinal flora can be crucial for the diseases.

UC is limited to the large intestine. It affects only the mucosa and submucosa, and often has visible blood in the intestine. In contrast, in Crohn's disease the whole intestinal wall is involved and in principle it can occur in the entire gastro-intestinal tract, but especially the last part of the small intestine is often affected.

### Autoantibodies/Antigens

There are two groups of antibodies that are disease specific and found in patients. One group recognizes self-antigens and the other group microorganism such as bacteria and fungi.
Most important antibodies are directed against the mannan polysaccharide in the cell membrane of *Saccharomyces cerevisiae* (ASCA).

In some cases anti-neutrophil cytoplasmic antibodies (ANCAs) appear. These antibodies are directed against cytoplasmic (C-ANCA) or perinuclear (P-ANCA) antigens of granulocytes or monocytes. Specific pattern can be identified by immunofluorescence staining of granulocytes. Only the atypical P-ANCA pattern is associated with IBD. It might be induced by cross-reaction with intestinal bacterial antigens, and histone 1, lactoferrin, cathepsin G and elastase are assumed to be target antigens, but both theories remain unproven.

### Diagnostic tests

Anti-*Saccharomyces cerevisiae* antibodies (ASCA) are predominantly found in patients with Crohn's disease (70%). Only 10% of patients with UC and 5% of healthy controls are positive for them.

The atypical P-ANCA pattern was found in 70% of UC cases and only 10% of Crohn's disease patients. Moreover, in less than 5% of non-inflammatory bowel diseases an atypical P-ANCA pattern is present. For that reason a combination of assays for these two antibodies is useful. In already identified IBD patients, a P-ANCA-negative/ASCA-positive result indicates Crohn's disease with a specificity of 95%, while P-ANCA-positive/ASCA-negative patients have UC with a specificity of 90%. However, these results show only 40–60% sensitivity.

# GASTROENTEROLOGY


## Kits

In our ***AESKULISA***® Gastroenterology panel we offer kits for cost-effective and reliable screening of anti-*Saccharomyces cerevisiae* IgA and IgG antibodies (ASCA) simultaneously (***AESKULISA***® Crohn's-Check). IgA and IgG antibodies can also be detected in a single test with high specificity.

For rapid and cost-effective screening of anti-neutrophil cytoplasmic antibodies (ANCAs), **AESKU.DIAGNOSTICS** offers ***AESKUSLIDES***® ANCA Granulocytes kits.

The ***AESKUBLOTS***® Gastro Pro assay is designed to assist in the diagnosis of celiac disease, pernicious anemia and Crohn's Disease. Celiac patients often have an IgA antibody deficiency, so in order to avoid false-negative results, ***AESKUBLOTS***® Gastro Pro detects IgA and IgG antibodies. Antigens coated are gliadin, neo-tTg, mannan (ASCA), parietal cell-antigen (PCA) and intrinsic factor.

## Pernicious Anemia

Pernicious anemia is a type of vitamin $B_{12}$ deficiency. Lack of vitamin $B_{12}$ results in a reduced number of red blood cells which starves the organs and tissue of oxygen. Common symptoms are tingling, tongue pain, pale skin, shortness of breath and fatigue. The prevalence is 0.1% of general population and 2% of people over 60 years. Pernicious anemia often co-occurs with other autoimmune diseases. Up to one-third of patients with autoimmune thyroid disease and 10% of patients with type 1 diabetes have pernicious anemia.

## Autoantibodies/Antigens

There are two main antibodies found in patients with pernicious anemia: anti-intrinsic factor antibodies and anti-parietal cell antibodies (APCAs). The intrinsic factor is needed for the absorption of vitamin $B_{12}$ and is produced by parietal cells in the gastric mucosa. In the case of APCA, it is assumed that there is a cross-reactivity of antibodies directed to *Helicobacter pylori* antigens. These antibodies may also recognize the alpha and beta subunits of the enzyme H+/K+-ATPase of parietal cells in stomach mucosa.

## Diagnostic tests

Initial blood tests are performed, which may show fewer red blood cells and low levels of vitamin $B_{12}$. High levels of homocysteine and methylmalonic acid (MMA) are also a sign of pernicious anemia, because vitamin $B_{12}$ is a necessary cofactor for their conversion into succinyl-CoA and methionine, respectively.

Anti-parietal cell antibodies (APCA) are sensitive for pernicious anemia and occur in 85% of patients. Also 10% of healthy controls and patients with other autoimmune diseases have them, so they are considered not specific, but very useful for screening.

In contrast, anti-intrinsic factor antibodies are highly specific for pernicious anemia, but less sensitive – 50% of patients have them. However, it is supposed that anti-intrinsic factor antibodies may disappear later in the disease process, so sensitivity may be higher at disease onset. In ambiguous cases a biopsy can be used for clarification.

## Kits

***AESKULISA***® Gastroenterology panel includes kits for the detection of anti-parietal cell antibodies and anti-intrinsic factor antibodies. ***AESKULISA***® parietal cell kit uses the native H+/K+ ATPase from porcine gastric mucosa as antigen and provides cost-effective and reliable screening. All assays are suitable for automation.

***AESKUSLIDES***® rat and mouse liver/kidney/stomach slides also allow the detection of antibodies against parietal cells (APCA) and their cost-effective screening.

***AESKUBLOTS***® Gastro Pro assay is designed to assist diagnosis of celiac disease, pernicious anemia and Crohn's Disease. Celiac patients often have an IgA antibody deficiency. In order to avoid false-negative results, ***AESKUBLOTS***® Gastro Pro detects IgA and IgG antibodies. Antigens coated are gliadin, neo-tTg, mannan (ASCA), parietal cell-antigen (PCA) and intrinsic factor.


| Ref No. | Product Name | Kit Configuration | Coated Antigen | Ig Class | Calibration/ Standard Range | Equivocal Zone/ Cut-off |
|---|---|---|---|---|---|---|
| *AUTOIMMUNITY* | | | | | | |
| *Gastroenterology* | | | | | | |
| | ***AESKULISA***® | | | | | |
| 3501 | Glia-A | Single-A | native alpha-gliadin | IgA | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3502 | Glia-G | Single-G | native alpha-gliadin | IgG | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3500 | Gliadin-Check | Single-Check | native alpha-gliadin | IgA + IgG | qualitative/ quantitative (0–300 U/ml) | Equivocal: 16–24 U/ml Cut-off: 20 U/ml |
| 3513 | DGP-A | Single-A | recombinant deamidated gliadin-peptides | IgA | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |

**References**

**1** Caja S., Mäki M., Kaukinen K., Lindfors K., Antibodies in celiac disease: implications beyond diagnostics. Cellular and Molecular Immunology, 2011;8(2):103-109, doi:10.1038/cmi.2010.65

**2** Mitsuyama K., Niwa M., Takedatsu H., et al. Antibody markers in the diagnosis of inflammatory bowel disease, World Journal of Gastroenterology, 2016;22(3):1304-1310, doi:10.3748/wjg.v22.i3.1304

**3** Minalyan A., Benhammou J. N., Artashesyan A., Lewis M. S., Pisegna J. R., Autoimmune atrophic gastritis: current perspectives, Clinical and Experimental Gastroenterology, 2017;2017(10),19-27

# GASTROENTEROLOGY *OVERVIEW*

| Ref No. | Product Name | Kit Configuration | Coated Antigen | Ig Class | Calibration/ Standard Range | Equivocal Zone/ Cut-off |
|---|---|---|---|---|---|---|
| *AUTOIMMUNITY* | | | | | | |
| *Gastroenterology* | | | | | | |
| | ***AESKULISA®*** | | | | | |
| 3514 | DGP-G | Single-G | recombinant deamidated gliadin-peptides | IgG | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3517 | DGP-GA | Single-GA | recombinant deamidated gliadin-peptides | IgG + IgA | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3515 | DGP-Check | Single-Check | recombinant deamidated gliadin-peptides | IgA + IgG | qualitative/ quantitative (0–300 U/ml) | Equivocal: 16–24 U/ml Cut-off: 20 U/ml |
| 3503 | tTg-A New Generation | Single-A | recombinant tissue transglutaminase (neo-epitopes), cross-linked with specific peptides of gliadin | IgA | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3504 | tTg-G New Generation | Single-G | recombinant tissue transglutaminase (neo-epitopes), cross-linked with specific peptides of gliadin | IgG | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3516 | tTg-GA New Generation | Single-GA | recombinant tissue transglutaminase (neo-epitopes), cross-linked with specific peptides of gliadin | IgA + IgG | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3510 | CeliCheck New Generation | Single-Check | recombinant tissue transglutaminase (neo-epitopes), cross-linked with specific peptides of gliadin | IgA + IgG | qualitative/ quantitative (0–300 U/ml) | Equivocal: 16–24 U/ml Cut-off: 20 U/ml |
| 3507 | ASCA-A | Single-A | recombinant purified mannan of saccharo-myces cerevisiae | IgA | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3508 | ASCA-G | Single-G | recombinant purified mannan of saccharo-myces cerevisiae | IgG | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3509 | Crohn´s-Check | Single-Check | recombinant purified mannan of saccharo-myces cerevisiae | IgA + IgG | qualitative/ quantitative (0–300 U/ml) | Equivocal: 16–24 U/ml Cut-off: 20 U/ml |
| 3512 | Intrinsic Factor | Single-G | recombinant intrinsic factor | IgG | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3511 | Parietal cell | Single-G | native H+/K+ ATPase from porcine gastric mucosa | IgG | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |

# Hemostasis

Hemostasis is a complex physiological process that stops bleeding at the site of injury while maintaining normal blood flow elsewhere in circulation. Balance between procoagulant and anticoagulant systems is critical for proper hemostasis. Imbalance leads to bleeding and thrombosis.

## Disease

Thrombophilia, is a multi-factor disease including several predisposing genetic and environmental risk factors. Abnormal blood coagulation increases the risk of thrombosis. Prominent genetic defects are protein C and protein S deficiency.

Protein C is an inhibitor of blood coagulation. Activated protein C inactivates activating factors Va and VIIIa by proteolytic cleavage, down-regulating thrombin generation. Absence of protein C results in increased clot formation. Protein C deficiency occurs in 0.2% of the general population.

Protein S is a cofactor in the activation of protein C. 60% of human protein S is bound to C4bP, a protein of the complement system, while the remaining 40% are free. Importantly, only free protein S is able to participate in the activation of protein C. Absence of protein S has similar results to protein C deficiency and the prevalence of protein S deficiency is 0.5%.

## Diagnostics

There are two main types of protein C and protein S assays, measuring activity and antigen. The activity assay is performed as an initial screening assay. If activity is decreased, an antigen assay is used to differentiate the subtype of deficiency. Antigen assays are immunoassays that detect the quantity of the antigen.

Protein C deficiency can be distinguished into two subtypes. In type I deficiency, normally functioning protein C molecules are produced in reduced quantity. In contrast, type II deficiency exhibits normal antigen quantity but with decreased activity level.

While protein S deficiency can be categorized as type I and type II, an additional subtype exists. Type III protein S deficiency is characterized by normal levels of total protein S, but low levels of free protein S associated with reduced protein S activity. Therefore, it is important to test for both total and free protein S antigen.

# HEMOSTASIS


**Kits**

**AESKU.DIAGNOSTICS** has developed the ***AESKULISA***® Hemostasis line for highly accurate and reliable determination of protein C and protein S antigen levels in human plasma. Deficient and normal plasma controls for protein C and protein S are included in the kits.

Free protein S can be detected by either the ***AESKULISA***® Protein S via precipitation of the C4b-bound protein S or directly with the ***AESKULISA***® Free Protein S assay.

| Ref No. | Product Name | Coated Antigen | Calibration/ Standard Range |
|---|---|---|---|
| *AUTOIMMUNITY* | | | |
| *Hemostasis* | | | |
| | ***AESKULISA***® | | |
| 3901 | Protein C | anti-human protein C antibody<br>International Standardization: NIBSC code 02/342 | quantitative (12.5–150%) |
| 3902 | Protein S | anti-human protein S and free protein S antibody<br>International Standardization: NIBSC code 02/228 | quantitative (12.5–150%) |
| 3903 | Free Protein S | anti-human free protein S antibody<br>International Standardization: NIBSC code 02/228 | quantitative (12.5–150%) |

**References**

**1** Dahlbäck B, Villoutreix BO. The anticoagulant protein C pathway. FEBS Lett, 2005;579(15):3310-3316

**2** Persson KE, Hillarp A, Dahlbäck B. Analytical considerations for free protein S assays in protein S deficiency. Thromb Haemost, 2001;86(5):1144-1147

**3** Wypasek E, Undas A. Protein C and protein S deficiency – practical diagnostic issues. Adv Clin Exp Med, 2013;22(4):459-467

# Miscellaneous

## Vitamin D

Vitamin D is an essential steroid hormone well known for its role in calcium homeostasis and bone metabolism. It is also involved in a number of physiological processes. There are two isomeric forms of Vitamin D, Vitamin D2 (Ergocalciferol) and Vitamin D3 (Cholecalciferol). While dietary sources like oily fish, egg yolk and fortified foods contain both forms, Vitamin D3 is also produced by the skin after sun exposure. In the liver, it is converted into 25-hydroxyvitamin D (25(OH)D), the major circulating form. Both Vitamin D and 25(OH)D are bound to Vitamin D binding protein (VDBP) in circulation. However, 25(OH)D is biologically inactive and has to be metabolized to its biologically active form 1,25-dihydroxy-vitamin D (1,25(OH)2D) in the kidneys by a tightly regulated mechanism.

### Disease

Insufficient levels of Vitamin D are associated with skeletal pathologies like rickets, osteoporosis and osteomalacia. Recent studies indicate a correlation of Vitamin D deficiency and a number of non-skeletal disorders including cardiovascular, autoimmune and infectious diseases, diabetes and cancer. Moreover, in pregnancy, a Vitamin D deficiency may predispose the fetus to develop chronic diseases. Approximately 1 billion people have Vitamin D levels below the normal range. People with limited sun exposure, elderly and infants are groups at risk for Vitamin D deficiency.

Vitamin D intoxication occurs very rarely but can lead to vascular and tissue calcification, with subsequent damage to the heart, blood vessels, and kidneys.

### Diagnostics

25(OH)D has the highest concentration of all vitamin D metabolites in the blood and its levels remain stable for almost 2 weeks. The serum level of 25(OH)D (representing D2 and/or D3) is widely accepted as useful biomarker for the determination of Vitamin D status and as a therapy control.

Methods like high-pressure liquid chromatography (HPLC), mass spectrometry (MS), radioimmunoassays (RIA), enzyme immunoassays (EIA), competitive protein binding assays (CPBA) and chemiluminescent immunoassays (CLIA) are routinely used for this measurement. Inter-methodology differences are frequent and therefore, use of an international standard is necessary to increase accuracy.

Compared to physical methods ELISA assays require low sample volumes and provide a fast, automation compatible and less laborious procedure.

# MISCELLANEOUS


**Kits**
***AESKULISA®*** 25OH Vitamin D allows a reliable and convenient quantitative determination of total 25-hydroxyvitamin D.

| Ref No. | Product Name | Kit Configuration | Coated Antigen | Ig Class | Calibration/ Standard Range | Equivocal Zone/ Cut-off |
|---|---|---|---|---|---|---|
| *AUTOIMMUNITY* | | | | | | |
| *Miscellaneous* | | | | | | |
| | ***AESKULISA®*** | | | | | |
| 350102 | Musk | Single | recombinant human musk | IgG | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 350103 | Titin | Single | recombinant human titin | IgG | qualitative/ quantitative (0–300 U/ml) | Equivocal: 12–18 U/ml Cut-off: 15 U/ml |
| 3801 | ß2-Micro-globulin | | polyclonal rabbit anti-beta-2-micro-globulin antibodies | | quantitative (0–12 µg/ml) | Cut-off: 3 µg/ml |
| 3810 | 25OH Vitamin D | | polyclonal anti-vitamin D antibodies | | quantitative (0–100 ng/ml) | |

**References**

**1** Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. Am J Clin Nutr. 2004;80(6Suppl):1678S-1688S

**2** Kennel KA, Drake MT, Hurley DL. Vitamin D deficiency in adults: when to test and how to treat. Mayo Clin Proc. 2010;85(8):752-757

**3** Zerwekh JE. Blood biomarkers of vitamin D status. Am J Clin Nutr. 2008;87(4):1087S-1091S

# PRODUCT HIGHLIGHTS

## **Highlights of *AESKULISA*®** Immunoassays for Diagnosis of Autoimmune Diseases

- High precision and reproducibility
- Highly sensitive & specific
- Profile or screening tests available
- Flexible choice of batch size
- Quantitative & qualitative determination possible
- More than 150 autoimmune parameters
- Cost-effective screening and confirmation

**AESKU**´s complete in-house production philosophy for ***AESKUBLOTS®*** products allows **AESKU** full control over the entire production chain, guaranteeing highest quality and unique product support.

Use of common reagents in all our autoimmune Immunoblots makes ***AESKUBLOTS®*** ideal for automation by saving space, cost and time with reduced loading and reagents' preparation.

For fast, easy and convenient automated processing and evaluation of ***AESKUBLOTS®*** the **HELIA®** system can be used.

> *__AESKUBLOTS®__ test line represents a variety of different immunoblots for efficient profile testing of autoimmune diseases.*

Easy semi-automatic processing is possible with **HELMED®** BLOT processors, the results can then be processed either manually or automatically with the **AESKU.SCAN** software.

# AESKUBLOTS®

## A STRATEGIC LINE-UP TO CHASE CLEAR RESULTS

# Kit Components


## Test principle

- Test strips are coated with different antigens forming lines.
- Serum sample from patients is added and during incubation, specific positive patient antibodies bind to the antigen. Afterwards, unbound substances are washed away.
- In a second incubation step, these specific antibodies are detected with an anti-human antibody linked to an enzyme (horseradish peroxidase, HRP). Afterwards, unbound antibody conjugates are washed away.
- After addition of the TMB*-substrate it is converted by an enzymatic reaction to a blue precipitate, appearing as a blue band on the strip. The reaction is stopped by distilled water.

## Features

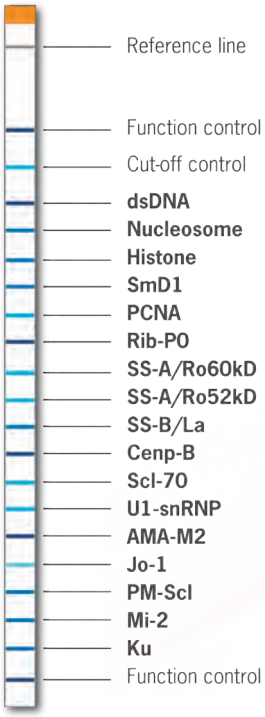
- 6 immunoblot panels according to clinical symptoms
- Up to 17 antigens on a blot strip
- Qualitative results
- Excellent correlation with ELISA and IFA

*3,3,5,5-Tetramethylbenzidin

# AESKUBLOTS® Autoimmunity line

**AESKUBLOTS®** Autoimmunity are fast, reliable and cost effective tools in the differential diagnosis of autoimmune diseases. They have been designed as screening tests and confirmatory tests after positive screening with IFA or ELISA. Striping of disease-specific antigens onto a nitrocellulose membrane permits the simultaneous determination of up to 17 different autoimmune antibodies in a single assay.

| Ref No. | Product Name | Tests/ Kit | Antibody Specifity | Ig Class | Calibration/ Standard Range |
|---|---|---|---|---|---|
| *AUTOIMMUNITY* | | | | | |
| | **AESKUBLOTS®** | | | | |
| 4001 | ANA-17 Pro | 24 tests | dsDNA, Nucleosomes, Histones, SmD1, PCNA, Rib-P0, SS-A/Ro60kD, SS-A/ Ro52kD, SS-B/La, Cenp-B, Scl-70, U1-snRNP, AMA-M2, Jo-1, PM-Scl, Mi-2, Ku | IgG | qualitative test |
| 4008 | ANA-17 comp | 24 tests | dsDNA, Nucleosomes, Histones, SmD1, PCNA, Rib-P0, SS-A/Ro60kD, SS-A/ Ro52kD, SS-B/La, Cenp-B, Scl-70, U1-snRNP, snRNP/Sm, Jo-1, PM/Scl, Mi-2, Ku | IgG | qualitative test |
| 4004 | Liver Pro | 24 tests | AMA-M2, Sp100, LKM-1, gp210, LC-1, SLA/LP | IgG | qualitative test |
| 4003 | Myositis Pro | 24 tests | Jo-1, Mi-2, PM-Scl, U1-snRNP, Ku | IgG | qualitative test |
| 4002 | Vasculitis Pro | 24 tests | PR3, MPO, GBM | IgG | qualitative test |
| 4005 | Gastro Pro | 24 tests | Gliadin, neo-tTg, mannan (ASCA), parietal cells (PCA), Intrinsic Factor | IgA + IgG | qualitative test |




# ***AESKUBLOTS®*** ANA-17 Pro

24 tests/kit

Conjugate: anti-human IgG-HRP

Color-coded (orange) test strips with cut-off and positive control

Immunoblot for qualitative detection of IgG antibodies against: dsDNA, nucleosomes, histones, SmD1, PCNA, Rib-P0, SS-A/Ro60kD, SS-A/Ro52kD, SS-B/La, Cenp-B, Scl-70, U1-snRNP, AMA-M2, Jo-1, PM-Scl, Ku and Mi-2 in human serum

***AESKUBLOTS®*** ANA-17 Pro is used for the differential diagnosis of systemic rheumatic diseases. Detection of autoantibodies in ***AESKUBLOTS®*** with corresponding specific antigens allows an easier and more reliable differentiation of specific ANAs (anti-nuclear antibodies). ANAs included occur in active and inactive systemic lupus erythematosus (SLE), mixed connective tissue diseases (MCTD), scleroderma, Sjogren's syndrome, primary biliary cirrhosis (PBC) and polymyositis. ANA-17 Pro antigens are fitted on the test strip according to their relevance for the individual autoimmune disease (SLE, Sjogren's syndrome, CREST syndrome, scleroderma, MCTD, myositis, and PBC) allowing easier interpretation.

# ***AESKUBLOTS®*** ANA-17 comp

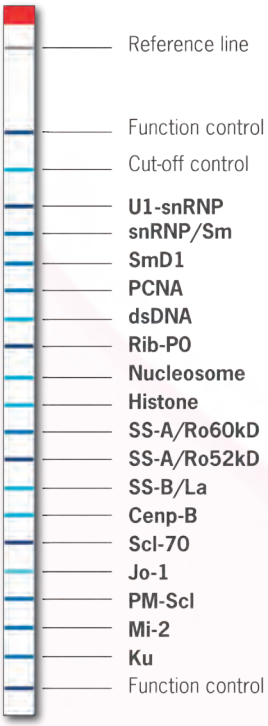
24 tests/kit

Conjugate: anti-human IgG-HRP

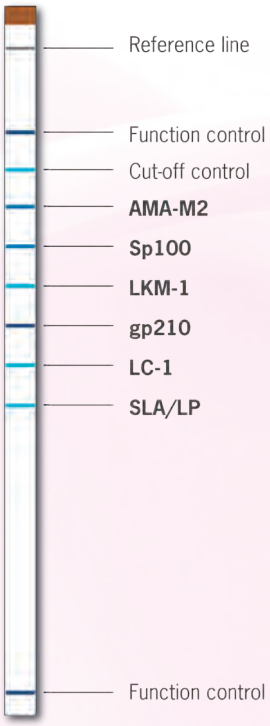
Color-coded (red) test strips with cut-off and positive control

Immunoblot for qualitative detection of IgG antibodies against: U1-snRNP, snRNP/Sm-complex, SmD1, PCNA, dsDNA, Rib-P0, nucleosomes, histones, SS-A/Ro60kD, A/Ro52kD, SS-B/La, Cenp-B, Scl-70, Jo-1, PM-Scl, Mi-2 and Ku in human serum

***AESKUBLOTS®*** ANA-17 comp is used for differential diagnosis of systemic rheumatic diseases. Detection of autoantibodies in ***AESKUBLOTS®*** with corresponding specific antigens allows an easier and more reliable differentiation of specific ANAs (anti-nuclear antibodies). ANAs included occur in active and inactive systemic lupus erythematosus (SLE), mixed connective tissue diseases (MCTD), scleroderma, Sjogren's syndrome, primary biliary cirrhosis (PBC) and polymyositis. ANA-17 comp antigens are fitted on the test strip according to their relevance for individual autoimmune disease (SLE, Sjogren's syndrome, CREST syndrome, scleroderma, MCTD, myositis, and PBC) allowing easier interpretation.



# AUTOIMMUNITY LINE




## ***AESKUBLOTS***® Liver Pro

24 tests/kit

Conjugate: anti-human IgG-HRP

Color-coded (brown) test strips with cut-off and positive control

Immunoblot for qualitative detection of IgG antibodies against: AMA-M2, Sp100, LKM-1, gp210, LC-1 and SLA/LP in human serum

***AESKUBLOTS***® Liver Pro is used as an aid in diagnosis of autoimmune liver diseases. Most important autoimmune liver diseases are autoimmune hepatitis (AIH) types 1–3, primary biliary cirrhosis (PBC) and a combination of both diseases (immunocholangiopathy). AIH is a chronic, progressive liver disease of unknown cause, responding well to immunosuppressive therapy, but if untreated has a poor prognosis. PBC is a chronic inflammatory disease of the small and medium-size bile ducts. Undetected it can lead to liver cirrhosis. An early and reliable diagnosis is therefore essential.

## ***AESKUBLOTS***® Myositis Pro

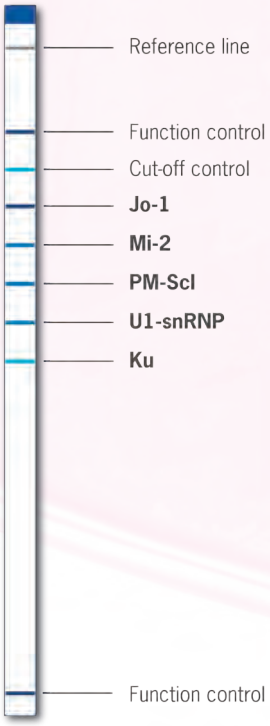
24 tests/kit

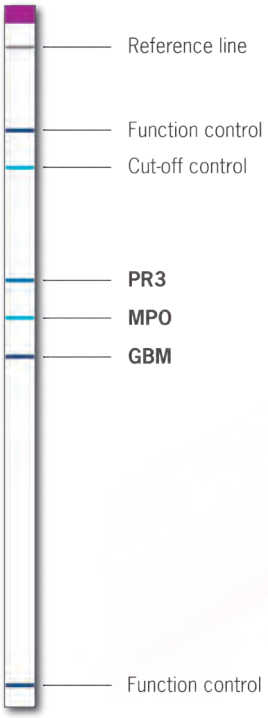
Conjugate: anti-human IgG-HRP

Color-coded (blue) test strips with cut-off and positive control

Immunoblot for qualitative detection of IgG antibodies against: Jo-1, Mi-2, PM-Scl, U1-snRNP and Ku in human serum

***AESKUBLOTS***® Myositis Pro is designed to help diagnosis of poly- and dermatomyositis, as well as myositis-associated autoimmune diseases. Autoimmune myositis represents a heterogeneous group of acquired muscle diseases. Their main clinical and morphological characteristics are muscle weakness and inflammatory infiltration of skeletal muscles.






## ***AESKUBLOTS***® Vasculitis Pro

24 tests/kit

Conjugate: anti-human IgG-HRP

Color-coded (purple) test strips with cut-off and positive control

Immunoblot for qualitative detection of IgG antibodies against: PR3, MPO and GBM in human serum

***AESKUBLOTS***® Vasculitis Pro is used for differential diagnosis of autoimmune vasculitis. Antibodies against proteinase 3 (PR3) and myeloperoxidase (MPO) belong to the group of anti-neutrophil cytoplasmic antibodies (ANCA), which have been described as important markers in differential diagnosis of autoimmune vasculitis. Anti-PR3 antibodies represent specific serological markers for granulomatosis with polyangiitis (Wegener's) and could play a role in pathogenesis. Anti-MPO antibodies occur in idiopathic and vasculitis-associated rapidly progressive glomerulonephritis, they are found in up to 70% of microscopic polyangiitis, and up to 5–50% in the Churg-Strauss syndrome. Serological detection of circulating antibodies against glomerular basement membrane in glomeruli (GBM) is the method of choice in diagnosis of Goodpasture's syndrome.

## ***AESKUBLOTS***® Gastro Pro

24 tests/kit

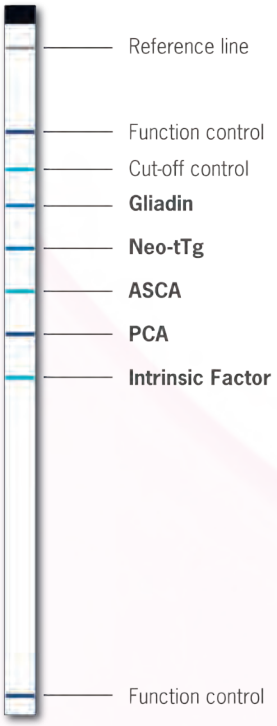
Conjugate: anti-human IgA-HRP and anti-human IgG-HRP

Color-coded (black) test strips with cut-off and positive control

Immunoblot for qualitative detection of IgA and IgG antibodies against: Gliadin, Neo-tTg, mannan (ASCA), parietal cells (PCA) and intrinsic factor-antigen

***AESKUBLOTS***® Gastro Pro is designed to assist in diagnosis of celiac disease, pernicious anemia and Crohn's Disease. Each section of gastro-intestinal tract can be affected by autoimmune gastro-intestinal diseases. Disease is often diagnosed years after initial symptoms, and outcome is severe in many cases.

Celiac patients often have an IgA deficiency. In order to avoid false-negative results, ***AESKUBLOTS***® Gastro Pro contains two separate conjugates to either IgA or IgG antibodies.



# PRODUCT HIGHLIGHTS

## **Highlights of *AESKUBLOTS*®** Immunoassay for Diagnosis of Autoimmune Diseases

- Variety of different immunoblots for major autoimmune diseases
- Multiplex testing with each reaction
- Cost-effective screening and differential diagnostic
- Minimal equipment needed
- Internal calibration included in every assay
- Positive control already included in every assay
- Automation friendly due to common reagents and protocols

Substrates in all our IFA kits are researched and developed in-house using the latest available technologies and are based on human substrates or native substrates, depending on optimal clinical correlation.

Different kit configurations provide the laboratory manager with maximum flexibility by allowing selection of best fitting formats their diagnostic needs.

All **AESKU.DIAGNOSTICS** kits have been designed consistently to use the same test procedure and common reagents, to simplify the complex autoimmune testing routine.

*Simplify your routine autoimmunity sample screening with **AESKUSLIDES®**. Indirect immunofluorescence assay (IFA) product line from **AESKU.GROUP** is designed for easy automation.*

***AESKUSLIDES®*** assay protocol files are available and have been validated by the **AESKU** QC department with **AESKU** systems semi- and full-automation.

# AESKUSLIDES®

## IFA PRODUCT LINE

# Kit Components


## Test principle

- Serum sample from patients is added and during incubation the specific antibody of positive patients binds to antigens presented by cells or tissue. Afterwards, unbound substances are washed away.
- In a second incubation step, these specific antibodies are detected with an anti-human antibody linked to a fluorochrome (fluorescein isothiocyanate, FITC). Afterwards, unbound antibody conjugates are washed away.
- Mounting medium is dispensed, covering each well.
- Slides are read under a Fluorescence microscope with FITC system, (490nm excitation filter, 510nm barrier filter).

# IMMUNOFLUORESCENCE TESTING

## Kit Configurations

| Antibody | | ANA | nDNA | ANCA | | AMA ASMA APCA | | | EMA |
|---|---|---|---|---|---|---|---|---|---|
| Substrate | | HEp-2 | *Crithidia luciliae* | Granulocytes | | Triple tissue LKS | | | Primate Esophagus |
| | | | | Ethanol | Formalin | Mouse (Separated) | Rat (Separated) | Rat (Wrapped) | |
| **Conjugate** | IgG | ● | ● | ● | ● | ● | ● | ● | ● |
| | IgA | | | | | | | | ● |
| **Slides** | 5 well | | | | | ● | ● | ● | ● |
| | 6 well | | | ● | ● | | | | |
| | 10 well | | ● | | | ● | ● | ● | ● |
| | 12 well | ● | | ● | ● | | | | |
| **Number of Tests / Kit** | 50 test | | | | | ● | ● | ● | ● |
| | 60 tests | | | ● | ● | | | | |
| | 100 test | | ● | | | ● | ● | ● | ● |
| | 120 tests | ● | | ● | ● | | | | |
| **Recommended Screening Dilution** | 1:5 | | | | | | | | ● |
| | 1:10 | | ● | | | | | | |
| | 1:20 | | | ● | ● | ● | ● | ● | |
| | 1:80 | ● | | | | | | | |

# ***AESKUSLIDES***® *THE IFA PRODUCT LINE*

All parameters from this product line are pre-programmed and validated for use in **HELMED**® and **HELIOS**® IFA processors.

Major patterns found on ***AESKUSLIDES***® are collected in the **HEL*PS+***® software and **HELIOS**® device software image library.

***AESKUSLIDES***® product line comprises a complete panel of cell and tissue based slide kits for detection of major autoantibodies.

Easy handling: ready to use reagents and controls vials fit directly in the **HELMED**® and **HELIOS**® racks.

Full traceability: every slide is labeled with a product barcode and unique number.

All components like wash buffer, sample buffer and mounting medium are common reagents amongst our entire product range.

Vacuum sealed slides provide long shelf life.

Specially formulated mounting medium allows its use in the **HELMED**® and **HELIOS**® IFA processors.

Strong hydrophobic Teflon mask coating of slides avoids cross contamination and requires minimal conjugate volumes.

***AESKUSLIDES***® product line includes a wide range of tissue and cell based slides for detection of major autoantibodies, supporting the fields of rheumatology (ANA HEp-2 and nDNA), angiology (ANCA), hepatology and gastroenterology (LKS triple tissue and EMA).

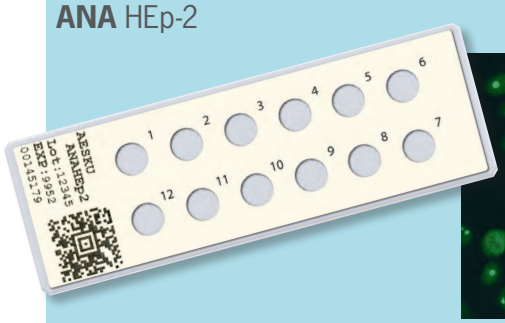
In Indirect Immunofluorescence Assay (IFA), tissue or cells are fixed on slides to present the antigen in an in-vitro like environment. Fluorescence assays are well suited for screening.

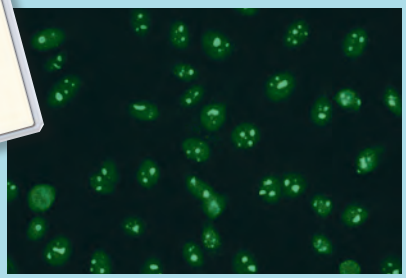
A pattern library is included in our **HEL*PS+***® software. This library provides assistance especially for new users to identify positive results. In addition, automated pattern recognition is possible with the **HELIOS**® device software.

All kits have been designed to use common reagents and same protocol, which were validated by **AESKU** QC division, simplifying your routine, and making processing easy to automate especially on **AESKU.SYSTEMS** semi-automated **HELMED**® IFA and automated **HELIOS**® analyzer.

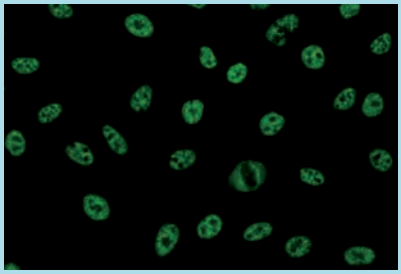
In addition, our kits include 10 or 50 slides with 5, 6, 10, or 12 wells, conjugate (anti-human-antibody-FITC-conjugate), positive as well as negative control, sample buffer, wash buffer and a unique mounting medium.

**ANA** HEp-2



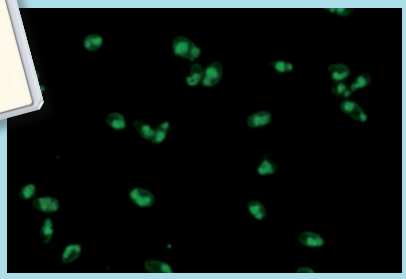



*Nucleolar*

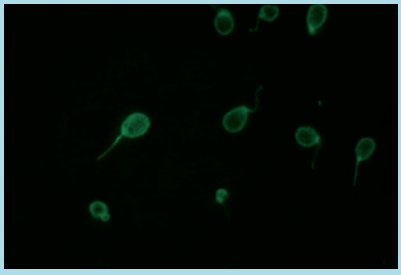


*Coarse speckled*

**nDNA** *Crithidia luciliae*

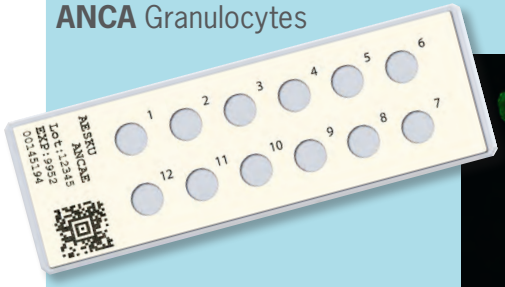
*Kinetoplast positive*

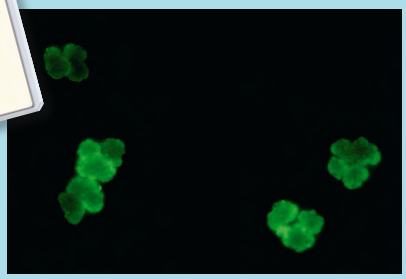


*Kinetoplast negative*

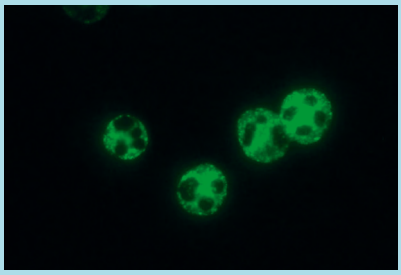
**ANCA** Granulocytes

*Available in two different fixations: Ethanol and Formalin*



*P-ANCA Ethanol*

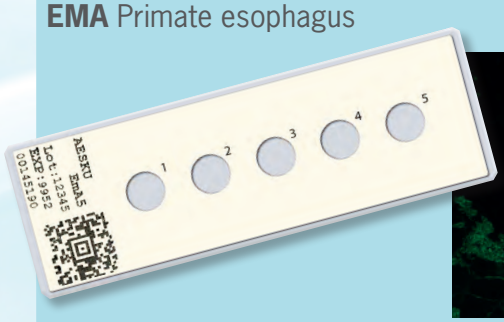


*C-ANCA Formalin*

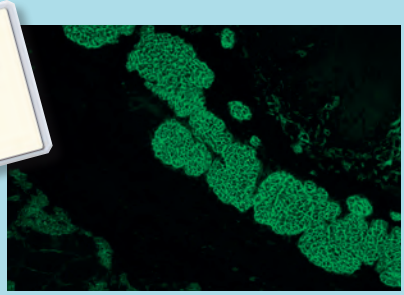
*Also available as 6 well slide*

# SUBSTRATES *OVERVIEW*

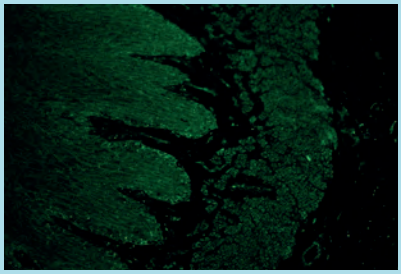
**EMA** Primate esophagus




*Also available as 10 well slide*

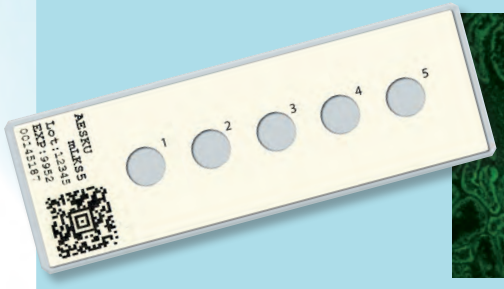


*Endomysium positive*

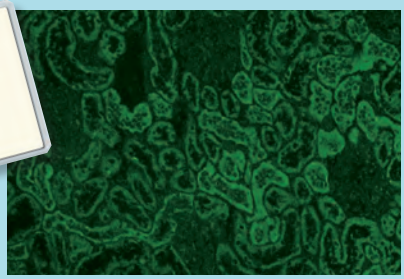


*Endomysium negative*

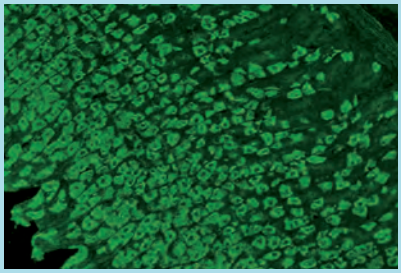
**AMA/ASMA/APCA** LKS (rat, mouse)




*Also available as 10 well slide*

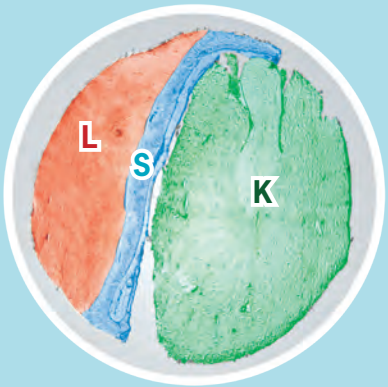


*AMA like pattern on kidney*



*Parietal cell positive on stomach*

***Triple tissues (LKS) are available in two different formats:***




***Separated***

**Liver**, **kidney** and **stomach** can be found in separated tissue sections.


***Wrapped***

**Liver** and **kidney** are wrapped with **stomach**.

# Kits

| Ref No. | Product name | Number of slides / Number of tests | For detection of | Ig Class | Substrate |
|---|---|---|---|---|---|
| *AUTOIMMUNITY* | | | | | |
| *ANA* | | | | | |
| | **AESKUSLIDES®** | | | | |
| 51.100 | ANA HEp-2 | 10x12 wells / 120 tests | Anti-nuclear antibodies (ANA) | IgG Fc (Fc gamma chain) | HEp-2 cells |
| 51.101 | ANA HEp-2 | 10x12 wells / 120 tests | Anti-nuclear antibodies (ANA) | IgG H + L (Heavy chain+ Light chain) | HEp-2 cells |
| 51.100 BULK 5 | ANA HEp-2 | 50x12 wells / 600 tests | Anti-nuclear antibodies (ANA) | IgG Fc (Fc gamma chain) | HEp-2 cells |
| 51.101 BULK 5 | ANA HEp-2 | 50x12 wells / 600 tests | Anti-nuclear antibodies (ANA) | IgG H + L (Heavy chain+ Light chain) | HEp-2 cells |
| *nDNA* | | | | | |
| | **AESKUSLIDES®** | | | | |
| 53.100 | nDNA | 10x10 wells / 100 tests | Anti-dsDNA antibodies (nDNA) | IgG | Crithidia luciliae |
| *ANCA* | | | | | |
| | **AESKUSLIDES®** | | | | |
| 54.100 | ANCA Ethanol | 10x12 wells / 120 tests | Anti-neutrophil cytoplasmic antibodies (ANCA) | IgG | Granulocytes |
| 54.101 | ANCA Formalin | 10x12 wells / 120 tests | Anti-neutrophil cytoplasmic antibodies (ANCA) | IgG | Granulocytes |
| *LKS* | | | | | |
| | **AESKUSLIDES®** | | | | |
| 517.050 | rLKS wrapped | 10x5 wells / 50 tests | Anti-mitochondrial antibodies (AMA) Anti-smooth muscle antibodies (ASMA) Anti-parietal cells antibodies (APCA) | IgG | Rat, Liver and Kidney wrapped in Stomach |
| 517.101 | rLKS wrapped | 10x10 wells / 100 tests | Anti-mitochondrial antibodies (AMA) Anti-smooth muscle antibodies (ASMA) Anti-parietal cells antibodies (APCA) | IgG | Rat-Liver and Kidney wrapped in Stomach |

# AUTOIMMUNITY LINE *OVERVIEW*

| Ref No. | Product name | Number of slides / Number of tests | For detection of | Ig Class | Substrate |
|---|---|---|---|---|---|
| **AUTOIMMUNITY** | | | | | |
| **LKS** | | | | | |
| | **AESKUSLIDES®** | | | | |
| 517.051 | rLKS separated | 10x5 wells / 50 tests | Anti-mitochondrial antibodies (AMA) Anti-smooth muscle antibodies (ASMA) Anti-parietal cells antibodies (APCA) | IgG | Rat-Liver, Kidney and Stomach |
| 517.100 | rLKS separated | 10x10 wells / 100 tests | Anti-mitochondrial antibodies (AMA) Anti-smooth muscle antibodies (ASMA) Anti-parietal cells antibodies (APCA) | IgG | Rat-Liver, Kidney and Stomach |
| 518.050 | mLKS separated | 10x5 wells / 50 tests | Anti-mitochondrial antibodies (AMA) Anti-smooth muscle antibodies (ASMA) Anti-parietal cells antibodies (APCA) | IgG | Mouse-Liver, Kidney and Stomach |
| 518.100 | mLKS separated | 10x10 wells / 100 tests | Anti-mitochondrial antibodies (AMA) Anti-smooth muscle antibodies (ASMA) Anti-parietal cells antibodies (APCA) | IgG | Mouse-Liver, Kidney and Stomach |
| **EMA** | | | | | |
| | **AESKUSLIDES®** | | | | |
| 512.050 | EMA | 10x5 wells / 50 tests | Anti-endomysium antibodies (EMA) | IgA | Primate Esophagus |
| 512.100 | EMA | 10x10 wells / 100 tests | Anti-endomysium antibodies (EMA) | IgA | Primate Esophagus |
| 512.060 | EMA | 10x5 wells / 50 tests | Anti-endomysium antibodies (EMA) | IgG | Primate Esophagus |
| 512.101 | EMA | 10x10 wells / 100 tests | Anti-endomysium antibodies (EMA) | IgG | Primate Esophagus |

# Slides

| Ref No. | Product name | Slide presentation | For detection of | Substrate |
|---|---|---|---|---|
| *AUTOIMMUNITY* | | | | |
| *ANA* | | | | |
| | ***AESKUSLIDES®*** | | | |
| S51.100 | ANA HEp-2 | 12 wells | Anti-nuclear antibodies (ANA) | HEp-2 cells |
| *nDNA* | | | | |
| | ***AESKUSLIDES®*** | | | |
| S53.100 | nDNA | 10 wells | Anti-dsDNA antibodies (nDNA) | Crithidia luciliae |
| *ANCA* | | | | |
| | ***AESKUSLIDES®*** | | | |
| S54.100 | ANCA Ethanol | 12 wells | Anti-neutrophil cytoplasmic antibodies (ANCA) | Granulocytes |
| S54.101 | ANCA Formalin | 12 wells | Anti-neutrophil cytoplasmic antibodies (ANCA) | Granulocytes |
| *LKS* | | | | |
| | ***AESKUSLIDES®*** | | | |
| S517.050 | rLKS wrapped | 5 wells | Anti-mitochondrial antibodies (AMA)<br>Anti-smooth muscle antibodies (ASMA)<br>Anti-parietal cells antibodies (APCA) | Rat-Liver and Kidney wrapped in Stomach |
| S517.101 | rLKS wrapped | 10 wells | Anti-mitochondrial antibodies (AMA)<br>Anti-smooth muscle antibodies (ASMA)<br>Anti-parietal cells antibodies (APCA) | Rat-Liver and Kidney wrapped in Stomach |
| S517.051 | rLKS separated | 5 wells | Anti-mitochondrial antibodies (AMA)<br>Anti-smooth muscle antibodies (ASMA)<br>Anti-parietal cells antibodies (APCA) | Rat-Liver, Kidney and Stomach |
| S517.100 | rLKS separated | 10 wells | Anti-mitochondrial antibodies (AMA)<br>Anti-smooth muscle antibodies (ASMA)<br>Anti-parietal cells antibodies (APCA) | Rat-Liver, Kidney and Stomach |
| S518.050 | mLKS separated | 5 wells | Anti-mitochondrial antibodies (AMA)<br>Anti-smooth muscle antibodies (ASMA)<br>Anti-parietal cells antibodies (APCA) | Mouse-Liver, Kidney and Stomach |
| S518.100 | mLKS separated | 10 wells | Anti-mitochondrial antibodies (AMA)<br>Anti-smooth muscle antibodies (ASMA)<br>Anti-parietal cells antibodies (APCA) | Mouse-Liver, Kidney and Stomach |
| *EMA* | | | | |
| | ***AESKUSLIDES®*** | | | |
| S512.050 | EMA | 5 wells | Anti-endomysium antibodies (EMA) | Primate Esophagus |
| S512.100 | EMA | 10 wells | Anti-endomysium antibodies (EMA) | Primate Esophagus |

# AUTOIMMUNITY LINE *OVERVIEW*

## Individual Components

| Ref No. | Product Name | Volume (ready to use) | For use with |
|---|---|---|---|
| **AUTOIMMUNITY** | | | |
| **Conjugate** | | | |
| | **AESKUSLIDES®** | | |
| C51.100 | Conjugate IgG ANA HEp-2 | 4 ml | ANA HEp-2 kits and slides IgG Fc (Fc gamma chain) |
| C51.100. BULK | Conjugate IgG ANA HEp-2 | 7.5 ml | ANA HEp-2 kits and slides IgG Fc (Fc gamma chain) |
| C51.101 | Conjugate IgG ANA HEp-2 | 4 ml | ANA HEp-2 kits and slides IgG H+L (Heavy chain+ Light chain) |
| C51.100. BULK | Conjugate IgG ANA HEp-2 | 7.5 ml | ANA HEp-2 kits and slides IgG H+L (Heavy chain+ Light chain) |
| C53.100 | Conjugate IgG nDNA | 4 ml | nDNA kits and slides |
| C54.050 | Conjugate IgG ANCA | 2 ml | ANCA Ethanol kits and slides |
| C54.051 | Conjugate IgG ANCA | 2 ml | ANCA Formalin kits and slides |
| C54.100 | Conjugate IgG ANCA | 4 ml | ANCA Ethanol kits and slides |
| C54.101 | Conjugate IgG ANCA | 4 ml | ANCA Formalin kits and slides |
| CDTIFA | Conjugate IgG LKS | 3.5 ml | all LKS kits and slides |
| C512.050 | Conjugate IgA EMA | 3.5 ml | EMA IgA kits and slides |
| C512.060 | Conjugate IgG EMA | 3.5 ml | EMA IgG kits and slides |
| **Controls** | | | |
| | **AESKUSLIDES®** | | |
| PC54.100 | C-ANCA pattern control | 0.5 ml | ANCA kits and slides |
| PC54.101 | P-ANCA pattern control | 0.5 ml | ANCA kits and slides |
| NCANCA | ANCA Negative control | 0.5 ml | ANCA kits and slides |
| PC51.100 | ANA Homogeneous control | 0.5 ml | ANA HEp-2 Kits and slides |
| PC53.100 | nDNA Positive control | 0.5 ml | nDNA kits and slides |
| PCDTIFA | AMA Positive Control | 0.5 ml | all LKS kits and slides |
| PC512.050 | EMA IgA Positive Control | 0.5 ml | EMA IgA kits and slides |
| PC512.060 | EMA IgG Positive Control | 0.5 ml | EMA IgG kits and slides |
| NCIFA | IFA Negative Control | 0.5 ml | ANA HEp-2, nDNA, LKS and EMA kits and slides |
| **Miscellaneous** | | | |
| | **AESKUSLIDES®** | | |
| MMIFA | Mounting Medium | 8 ml | all ***AESKUSLIDES®*** |
| MMIFA .BULK | Mounting Medium | 10 ml | all ***AESKUSLIDES®*** |
| EBIFA | Counterstain (Evans Blue) | 3 ml | all ***AESKUSLIDES®*** |
| SBIFA | Sample Buffer | 70 ml | all ***AESKUSLIDES®*** |
| WBIFA | Wash Buffer | 100 ml *(10x concentrated)* | all ***AESKUSLIDES®*** |

## Further reading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# PRODUCT HIGHLIGHTS

## Highlights of ***AESKUSLIDES®*** *AUTOIMMUNITY LINE*

- Wide range of tissue and cell-based slides for detection of major autoantibodies
- Vacuum sealed slides for long shelf life
- Traceability, due to barcoded slides and unique numbers
- All major routine parameters available
- High sensitivity
- Automation friendly due to common reagents and protocols
- Easy handling: Vials fit directly in the **HELMED®** and **HELIOS®**
- Suitable for automated reading with **HELIOS®**, due to unique mounting medium
- Pattern library for better evaluation

# NOTES

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