



IMMUNOBLOTS

A MAINSTAY OF MULTIPARAMETRIC ANTIBODY ANALYSIS

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The spectrum of disease-associated antibodies for laboratory testing is constantly growing thanks to the continual identification of novel target antigens. This poses the question of how many, and which antibodies to investigate, in a particular disease suspicion. In many of these instances immunoblots are a useful analysis method, as they can include large panels of antigens. Up to 54 antibodies can easily be investigated in parallel.

Line blots composed of individual membrane chips offer the additional benefit of allowing antigens with widely differing properties to be combined on one test strip. Thus, profiles can be composed according to the disease application, regardless of the antigens involved. The antigen preparations and membrane fragments are individually optimised to

provide maximum antibody detection efficiency for each parameter.

Comprehensive immunoblot profiles support diagnosis of conditions as diverse as autoimmune liver diseases, myositis, systemic sclerosis, neurological diseases, rheumatic diseases and allergies (see *table 1*). They are also used for diagnosis of infections such as *Borrelia*, Epstein-Barr virus and hantaviruses and for determination of the immune status in pregnancy. In particular, many profiles in the EUROLINE series provide parameter combinations that are not available in any other format and include antigens that are exclusive to this test method. This wide antigen scope even allows identification of patients with rare antibody constellations. These are some examples of how immunoblot analyses are employed to help diagnose and differentiate various diseases:

AUTOIMMUNE LIVER DISEASES

Primary biliary cirrhosis (PBC) is an immune-mediated chronic inflammatory cholestatic liver disease characterised by autoantibodies directed against mitochondrial, nuclear dot and nuclear membrane antigens. Immunoblot analysis with 14 relevant antigens (see *figure 1*) aids the diagnosis of PBC, its differentiation from autoimmune hepatitis (AIH) and the detection of overlap syndromes.

The main serological marker for PBC is autoantibodies against the mitochondrial antigen M2 (AMA-M2). The majority of these autoantibodies are directed against the enzyme pyruvate dehydrogenase. However, 5-10% of patients exhibit only antibodies against the two other main autoantigens of the M2 complex, branched-chain 2-oxoacid dehydrogenase and oxoglutarate dehydrogenase. A designer fusion protein comprising the immunogenic domains of all three enzyme complexes increases the sensitivity for AMA-M2 by detecting all three types of antibody simultaneously.

Further PBC-specific antigens are gp210 (glycoprotein 210), Sp100 (spot-pattern 100 kDa protein) and PML (promyelocytic leukaemia protein). Antibodies against SLA/LP (soluble liver antigen/liver pancreas antigen), LKM-1 (liver-kidney microsomes) and LC-1 (cytosolic liver antigen type 1), on the other hand, are characteristic for AIH and enable immediate discrimination of PBC and AIH. The antigens SS-A, Ro-52, Scl-70, CENP A, CENP B, PGDH are relevant for the identification of overlap syndromes in autoimmune liver diseases, for example with Sjögren's syndrome.

In a clinical study, sera from patients with clinically characterised PBC or viral hepatitis, and healthy blood donors were analysed using line blot. The immunoblot provided an unrivalled overall sensitivity for PBC of 94% at a specificity of 99%. The M2 antigen, in particular M2-3E, contributed the most to the sensitivity, while in 6% of cases immune reactivities were directed exclusively against Sp100, PML and/or gp210. This study emphasises the benefits of multiparametric testing for identifying patients who might otherwise be overlooked with a narrower analysis.

In AIH patient sera from four centres worldwide, the prevalence of anti-SLA/LP positivity was found to be 5-19% depending on the region. Anti-SLA/LP and anti-LC-1 antibodies show 100% specificity for AIH, whereas anti-LKM-1 occur in both AIH and viral hepatitis.

In a further study anti-PDGF antibodies were found in 23% of samples from AIH patients and 9% of PBC samples, but not in any sera from patients with viral hepatitis. And in a prospective study in a clinical immunological laboratory, antibodies against SS-A, Scl-70, CENP A and CENP B were found in 13%, 3%, 6% and 10% respectively, of samples that had been submitted for investigation of specific autoantibodies in PBC and AIH. Thus, extending the scope of the analysis with these additional antigens yielded useful additional information for diagnosis.

PARANEOPLASTIC SYNDROMES

Paraneoplastic syndromes (PNS) are neurological syndromes accompanying malignant tumours, for example small-cell lung carcinoma or mammary carcinoma. Neuronal antigens expressed from the tumour cells induce the formation of autoantibodies, which trigger immune reactions leading to the neurological disorder. Autoantibody detection can aid the diagnosis of PNS and may provide the first evidence of an underlying tumour. Antibodies against neuronal antigens are determined by indirect immunofluorescence and blot techniques.

An immunoblot containing 12 of the most important neuronal antigens (see *figure 1*) combines highly sensitive and specific antibody detection with simple evaluation. The test includes the recombinant neuronal antigens amphiphysin, CV2, PNMA2 (Ma2/Ta), Ri, Yo and Hu, recoverin, SOX1, titin, Zic4, GAD65 and Tr (DNER). In studies

with various patient panels the specificity for all antigens was more than 99%, while the sensitivity amounted to 100% for all antigens except PNMA2 (89%), GAD65 (86%) and Tr (93%). In combination with indirect immunofluorescence this profile ensures highly efficient detection of autoantibodies in PNS.

MYOSITIS

Polymyositis is an inflammatory disease of skeletal muscle with perivascular lymphocytic infiltration. When the skin is additionally involved the disease is known as dermatomyositis. The detection of specific autoantibodies is essential for diagnosis and differentiation of these two diseases and identification of overlap syndromes. Since the presence of isolated individual autoantibodies is typical, multiparametric testing is a prerequisite.

A unique 15-antigen immunoblot profile focuses exclusively on autoantibodies in autoimmune inflammatory myopathies (see *figure 1*). It includes the nuclear antigens Mi-2a, Mi-2b, Ku, PM-Scl75, PM-Scl100, SAE1, NXP2, MDA5 and TIF1Y and the cytoplasmic antigens Jo-1 (histidyl-tRNA synthetase), PL-7 (threonyl-tRNA synthetase), PL-12 (alanyl-tRNA synthetase), EJ (glycyl-tRNA synthetase), OJ (isoleucyl-tRNA synthetase) and signal recognition particle (SRP). In sera from myositis patients, the individual test parameters yielded prevalence ranging from 1% for the rare anti-EJ and -OJ to 21% for anti-Jo-1. The overall serological detection rate for myositis was typically 26-37%. The specificities for the individual antigens lay between 95-100%. As the first test to provide a profile of myositis-associated antigens with the ease of interpretation of a line blot, this immunoblot represents a powerful new tool for myositis diagnostics.

SYSTEMIC SCLEROSIS

Systemic sclerosis (SSc) is a rare and very heterogeneous disease, which is characterised by various autoantibodies, of which antibodies against Scl-70 and against centromeres play a major role. Further autoantibodies, for example against PM-Scl75 and against PM-Scl100, have recently been shown to be reliable markers for SSc. A dedicated immunoblot profile provides parallel detection of 12 SSc-associated autoantibodies (see *figure 1*), namely Scl-70, CENP A, CENP B, RNA polymerase III subunits RP 11 and RP155, fibrillarin, NOR-90, Th/To, PM-Scl100, PM-Scl75, Ku and PDGF receptor. Ro-52 is also included to provide additional diagnostic information, although anti-Ro-52 antibodies are not specific for SSc. With the most extensive range of SSc-associated antigens available in one test, this assay is highly suited as a first-line screening test for suspected cases of SSc.

In two clinical studies performed using a total of 331 or 208 sera from patients with characterised SSc, patients with other diseases and healthy blood donors, the overall detection rate for SSc using the line blot amounted to 85% or 77%, respectively. The specificities for the individual antigens were all greater than 97%. A large proportion (89%) of the SSc sera showed an isolated positive reaction with just one antigen, most frequently centromeres (48%), Scl-70 (24%) or RNA polymerase III (17%), highlighting the importance of multiparametric analysis for SSc diagnostics.

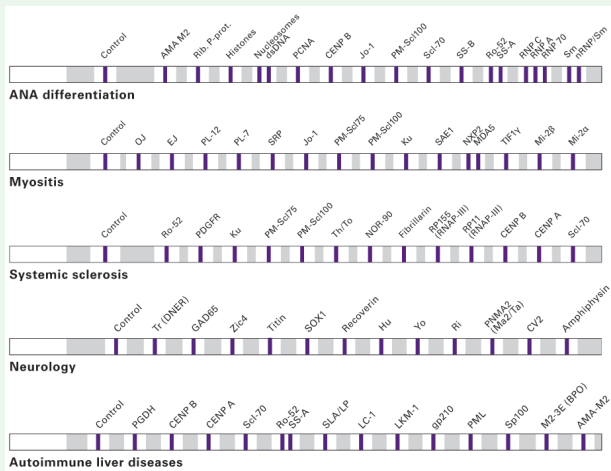
ANA DIFFERENTIATION

The detection of antibodies against nuclear antigens (ANA) aids the identification and differentiation of various rheumatic diseases, for example systemic lupus erythematosus (SLE), Sjögren's syndrome (SS), Sharp syndrome (mixed connective tissue disease, MCTD), etc. Sera are initially screened by indirect immunofluorescence using HEp-2 cells and primate liver, and results are subsequently confirmed and differentiated using specific tests.

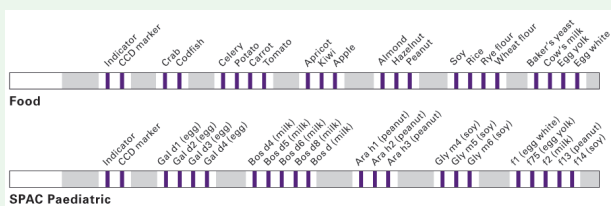
Line blots are a popular choice for ANA differentiation due to their extensive combinations of antigens. The most comprehensive →

immunoblot ANA characterisation is provided by a profile containing a total of 18 antigens (see figure 1), namely nRNP/Sm, Sm, RNP 70, RNP A, RNP C, SS-A, Ro-52, SS-B, Scl-70, PM-Scl, Jo-1, CENP B, PCNA, dsDNA, nucleosomes (highly purified), histones, ribosomal P-proteins and AMA M2.

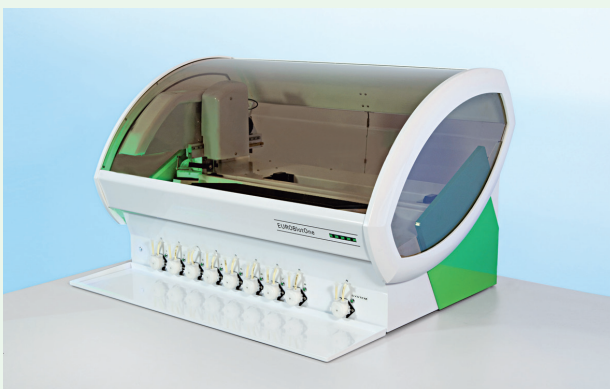
▼FIGURE 1: Profiles for autoimmune diagnostics



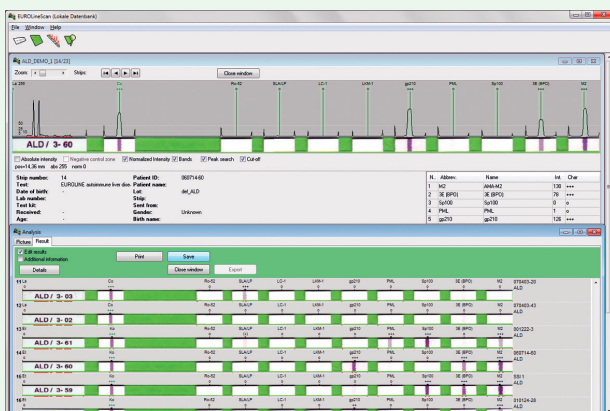
▼FIGURE 2: Examples of profiles for allergy diagnostics



▼FIGURE 3: EUROBlotOne for fully automated blot processing



▼FIGURE 4: EUROLineScan evaluation software



ALLERGY DIAGNOSTICS

Multiparametric immunoblots also play an important role in allergy diagnostics. For example, specific IgE antibodies against up to 54 allergens can be analysed in parallel. The immunoblots are composed of purified allergen extracts and single purified allergen components (SPAC). The defined allergenic proteins, in particular, deliver precise, in-depth information about the source of sensitisation. The identification of the exact disease-causing allergens allows allergologists to assess the risk of cross-reactions and determine patients' suitability for specific immunotherapy.

Profiles are available for the determination of IgE antibodies against a range of parameters encompassing inhalation, food and insect venom allergies, atopy, cross-reactions and paediatrics. Each profile is tailored to address a specific region or diagnostic query (see figure 2). The inclusion of a band of cross-reactive carbohydrate determinant (CCD) on each strip aids interpretation of the relevance of the specific IgE reactions.

ADDED EFFICIENCY THROUGH AUTOMATION

The efficiency and ease of multiparametric immunoblot analyses can be increased further through automation of the entire procedure. Modern immunoblot processing systems such as the EUROBlotOne (see figure 3) provide complete automation of all steps, from sample entry to report release, freeing up valuable time for laboratory staff and increasing reliability. Up to 44 strips can be incubated per run and different tests can be combined in one run. Sample mix-ups are avoided through use of an integrated barcode scanner. Specialised software (EUROLineScan) automatically identifies, quantifies and assigns the bands and generates the patient reports (see figure 4). Administration and storage of data by the automated system eliminates the need to store potentially infectious blot strips.

SUMMARY

Immunoblots enrich the laboratory's test portfolio with their broad antigen combinations, easy interpretation and automatability. By employing comprehensive disease-oriented profiles, clinicians can harvest extensive diagnostic information for minimal effort. As the repertoire of test parameters grows, immunoblots can be easily supplemented with newly identified antigens. This versatility will ensure that immunoblots remain a mainstay of the diagnostic laboratory. 📄

▼TABLE 1: Scope of multiparametric immunoblot analyses

Disease profile(s)	No. of antigens	Characteristics
Autoimmune liver diseases	14	PBC and AIH specific
Paraneoplastic syndromes	12	Exclusively PNS specific
Myositis	15	Exclusively myositis specific
Systemic sclerosis	12	Exclusively SSc specific
ANA differentiation	18	Markers for different rheumatic diseases
Allergy	up to 54	SPAC and allergen extracts
Borrelia	13	Early- and late-stage markers
Bordetella pertussis	3	Infection/vaccination differentiation
Epstein-Barr virus	5	Early- and late-stage markers
Hantaviruses	6	Different global serotypes
Parvovirus B19	4	Early- and late-stage markers
TO.R.C.H.	10	High-risk infections in pregnancy