Antinuclear antibodies (ANA)
Antibodies to extractable nuclear antigens (ENA)

Enzyme immunoassays for the diagnostics of systemic autoimmune diseases

ELISA, **IMMUNOBLOT**, and **MICROBLOT-ARRAY** kits are optimized and validated for detection of IgG antibodies in human serum and plasma

CE IVD

Diagnostic kits are intended for professional use in the laboratory.

BG TestLine®

Introduction

Determination of antinuclear antibodies is important for diagnostics of systemic autoimmune diseases. These organ nonspecific auto-antibodies are directed to intracellular antigens located mainly in the nucleus of the cell. Their detection can indicate the presence of some systemic autoimmunopathologic process – especially: **systemic lupus erythematodes (SLE), Sjögren's syndrome (SS), scleroderma, mixed connective tissue disease (MCTD), systemic sclerosis, polymyositis and dermatomyositis**.

An important group of antinuclear antibodies represents antibodies against ENA (extractable nuclear antigens: SS-A/Ro, SS-B/La, Sm, RNP, Scl-70 and Jo-1). They are mainly ribonucleoproteins and nuclear enzymes.

Identification of single auto-antibody specificity is an important tool for differential diagnosis of systemic auto-immune diseases.

Antibodies against SS-A/Ro and SS-B/La often occur in patients with SS and SLE. They can be also found in mothers of children with neonatal lupus and congenital heart block. Anti-Sm antibodies represent a highly specific marker and one of diagnostic and classification criteria for SLE. Also anti-RNP antibodies (a part of Sm/RNP complex) are often detected in patients with SLE. Presence of these antibodies is highly specific for MCTD (particularly when anti-Sm antibodies are missing). Detection of anti-Jo-1 antibodies is significant for another group of organ non-specific autoimmune diseases – myositides. Antibodies against antigen Scl-70 and centromere B are typical for diagnosis of systemic sclerosis (particularly its progressive forms).

The group of antinuclear antibodies also includes antibodies against nucleic acids (ssDNA, dsDNA), complexes of nuclear proteins (DNP, RNP) and histones.

Antibodies to double-stranded DNA (anti-dsDNA) fall in the group of antinuclear antibodies. This is a heterogeneous group of antibodies that are directed against various epitopes on a native double-stranded DNA molecule. The antibodies are considered highly specific for systemic lupus erythematosus (SLE).

Examination of anti-nuclear antibodies may also be included the diagnosis process for other autoimmune diseases, such as primary biliary cirrhosis.

The diagnostic process of autoimmune systemic diseases is also enhanced by the detection of anti-DFS70 antibodies, which are common in healthy subjects but rarely occur in patients with systemic autoimmune rheumatic diseases.

Antibody prevalence in relation to individual diseaeses

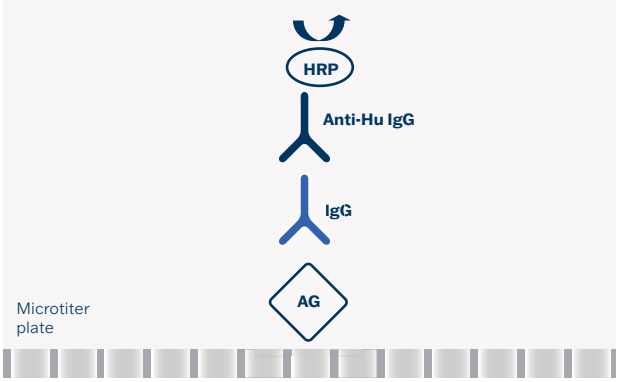
Antigen	Description	Probable association with disease			
		ANA	Myositis	Scleroderma	SLE and other connective tissue diseases
Jo-1	Hystidyl tRNA synthetase	●	●		
PL-7	Threonyl tRNA synthetase	●	●		
PL-12	Alanyl tRNA synthetase	●	●		
EJ	Glycyl tRNA synthetase	●	●		
OJ	Isoleucyl tRNA synthetase	●	●		
KS	Asparaginyl tRNA synthetase	●	●		
YARS	Tyrosyl tRNA synthetase (Ha)	●	●		
ZoA	Phenylalanyl tRNA synthetase	●	●		
ZoB	Phenylalanyl tRNA synthetase	●	●		
HMGCR*	3-hydroxy-3methylglutaryl-coenzyme A reductase	●	●		
SAE-1	Small ubiquitin-like modifier activating enzyme	●	●		
SAE-2	Small ubiquitin-like modifier activating enzyme	●	●		
SRP54	Signal recognition particle	●	●		
Mi-2	Helicase protein-nuclear transcription	●	●		
TIF1γ	Transcription Intermediary Factor 1	●	●		
MDA5	Melanoma differentiation associated protein 5 (CADM-140)	●	●		
NXP2	Nuclear matrix protein 2 (p140, MJ)	●	●		
PMScl 100	Human exosome complex	●	●	●	
PMScl 75	Human exosome complex	●	●	●	
Scl70	DNA-topoisomerase I	●		●	
CENP A	Centromere A	●		●	
CENP B	Centromere B	●		●	
POLR3A	RNA polymerase III	●		●	
NOR90	Nucleolar transcription factor 1 (Ubtf1)	●		●	●
Th/To	Ribonuclease P protein subunit 25 (Rpp25)	●		●	
PDGFR-β	Platelet-derived growth factor receptor beta	●		●	
Fibrillarin	U3 RNP – fibrillarin	●		●	
Ro52	TRIM21	●	●	●	●
Ro60	Sjögren's-syndrome-related antigen A (SS-A)	●			●
La	Sjögren's-syndrome-related antigen B (SS-B)	●			●
RNP A	U1 small nuclear ribonucleoprotein A	●		●	●
RNP 68/70	U1 small nuclear ribonucleoprotein 68/70 kDa	●		●	●
RNP C	U1 small nuclear ribonucleoprotein C	●		●	●
SmB	Smith antigen B	●			●
SmD	Smith antigen D	●			●
PCNA	Proliferating cell nuclear antigen	●			●
P0	Ribosomal protein P0	●			●
Ku	Ku (p70/p80)	●	●	●	●
Nucleolin	Nucleolin	●			●
Histons	Histone	●			●
Nucleosome	Nucleosome	●			●
dsDNA	Double-stranded DNA	●			●
M2	Mitochondrial M2 (AMA-M2)	●		●	
DFS70	Dense fine speckled 70 antigen	●			

*Check availability in your country

ELISA

Test Principle

The assays are based on a sandwich type of ELISA method.



User Comfort

- Ready-to-use components
- Colour-coded components
- Breakable colour-coded microplate strips CUT-OFF included
- Semi-quantitative evaluation of results (Index of positivity) for ENA Screen and ENA profile
- Quantitative evaluation for individual antigen assays (U/ml, IU/ml for dsDNA), calibrators included
- Easy assay procedure

Advantages

- High diagnostic specificity and sensitivity
- High reproducibility
- High dynamics of antibody response
- Total screening time 1,5 hours
- Ready for automation
- Customer support

Antigens

EIA ENA screen plus – mixture of native and recombinant antigens Ro52/SS-A, Ro60/SS-A, La/SS-B, Sm, RNP-A, RNP-C, RNP 68, Scl-70, Jo-1 and CENPB

EIA ENA profile plus – antigens Ro52/SS-A, Ro60/SS-A, La/SS-B, Sm, RNP-A, RNP-C, RNP 68, Scl-70 and Jo-1, CENP A and B

EIA dsDNA – purified native human dsDNA

EIA Scl-70 –mixture of native and recombinant Scl-70 antigens

EIA Sm – highly purified native Sm antigen

EIA SS-A – highly purified native antigen SS-A/Ro60 (60 kDa) and recombinant antigen SS-A/Ro52 (52 kDa)

EIA SS-B – recombinant SS-B/La antigen

EIA U1RNP – mixture of recombinant RNP A, RNP C and RNP 68 antigens

Protocol Summary

Step		Test steps
	1.	Dilution of samples – serum/plasma 1:101 (10 µl + 1 ml)
	2.	Pipette Controls and diluted samples 100 µl – Including blank
	3.	Incubate 30 min. at 37 °C
	4.	Aspirate and wash the wells 5 times
	5.	Add Conjugate 100 µl – Including blank
	6.	Incubate 30 min. at 37 °C
	7.	Aspirate and wash the wells 5 times
	8.	Add 100 µl Substrate (TMB-Complete) – Including blank
	9.	Incubate 15 min. at 37 °C
	10.	Add 100 µl Stopping solution – Including blank
	11.	Read colour intensity at 450 nm

Clinical Application

- Screening test for the detection of antibodies against ENA in systemic connective tissue diseases
- Tests to detect antibodies against individual ENAs
- Differential diagnosis of systemic autoimmune disease

Test Characteristics

ELISA	Diagnostic sensitivity	Diagnostic specificity
EIA ENA screen plus	96.2%	97.7%
EIA ENA profile plus	95.0%	99.0%
EIA dsDNA	97,8%	98,7%
EIA Jo-1	95.5%	97.9%
EIA Scl-70	97.9%	97.9%
EIA Sm	95.7%	98.2%
EIA SS-A	95.8%	97.5%
EIA SS-B	97.9%	97.9%
EIA U1RNP	97.7%	98.2%

Clinical Data

Clinical sample testing results – EIA ENA screen plus

Patients with systemic autoimmune disease (n=143)

Diagnosis	Tested (n)	Positive (n)	% reaction
SLE	65	63	97 %
Sjögren's syndrom	13	12	92 %
Sclerodermia	15	14	93 %
Dermatomyositis	11	11	100 %
Raynaud's syndrome	6	6	100%
Unclear systemic autoimmune disease	33	29	88 %

Blood donors (n=227) – control group

Positive	3	1 %
Negative	224	99 %

Clinical sample testing results – EIA ENA profile plus

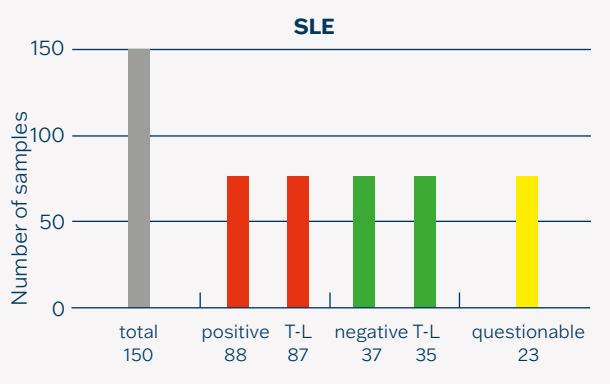
SLE – Systemic lupus erythematodes	79
SSc – Systemic scleroderma	72
DM – Dermatomyositis	23
Blood donors	50
Total	**224**

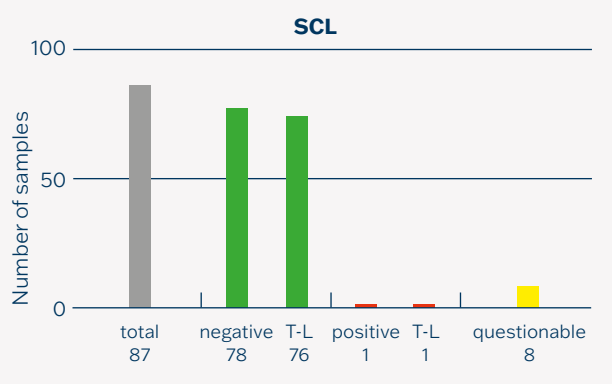
Results achieved on the test population

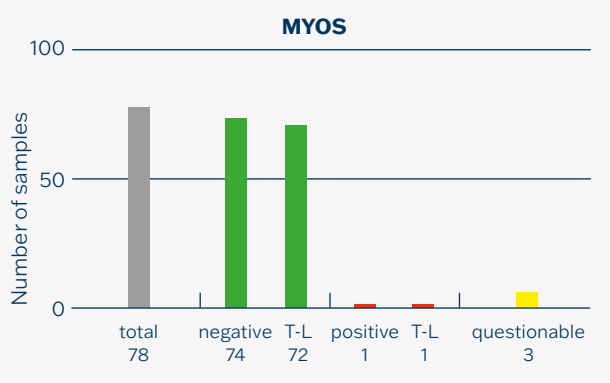
EIA ENA profile plus

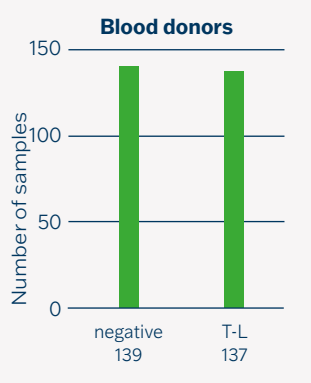
Antigen	Sensitivity	Specificity
CENP	100 %	98 %
Jo-1	100 %	100 %
Scl-70	100 %	100 %
Sm	90 %	97 %
SS-A	97 %	99 %
SS-B	85 %	98 %
U1RNP	100 %	98 %

Results of testing clinical samples – dsDNA EIA



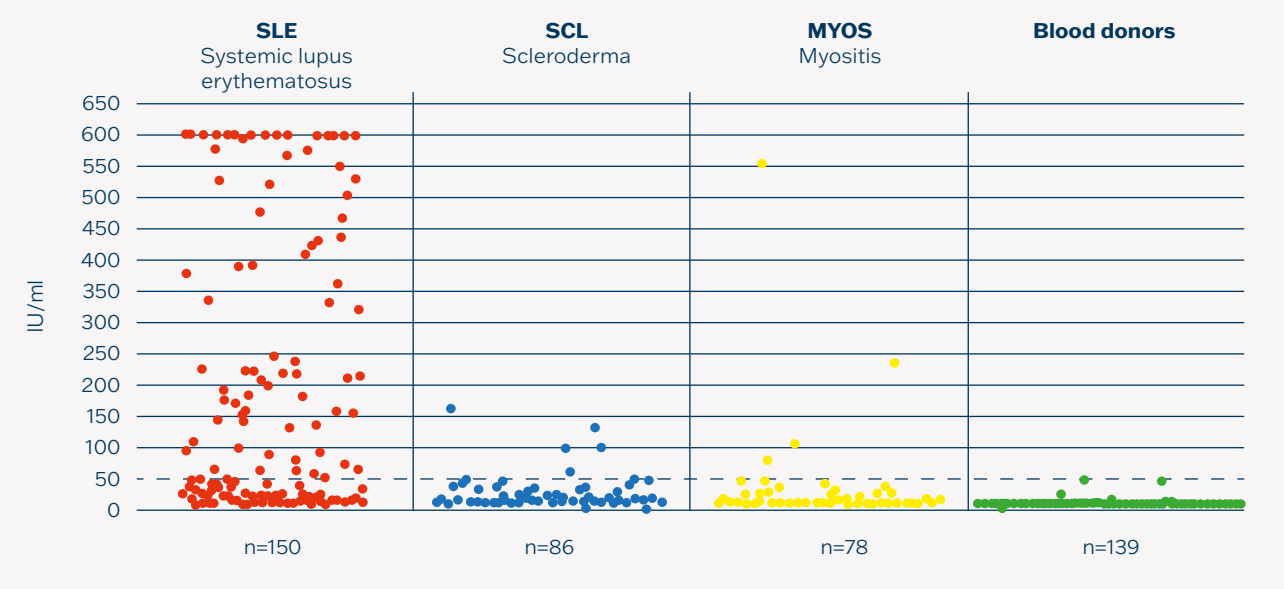






- Positive – result confirmed by two different ELISA kits
- Negative – result confirmed by two different ELISA kits
- Questionable – an agreement was not reached for two different EIA kits

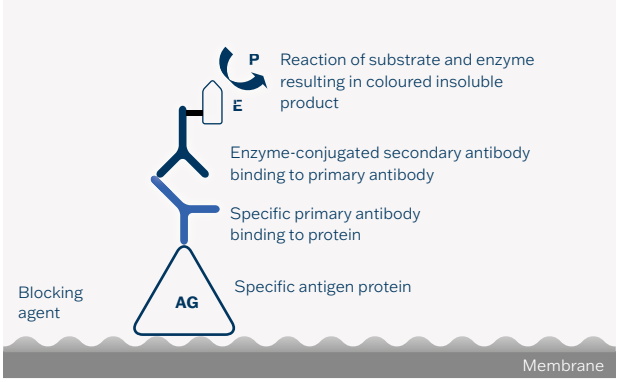
Antibody response dynamics – dsDNA EIA



IMMUNOBLOT

Test Principle

Antigens are transferred to a nitrocellulose membrane using a micro-dispensing method.



User Comfort

- Ready-to-use components
- Colour-coded strips
- Interchangeable components
- Positive and Negative controls
- Control line on the strip
- Easy assay procedure

Advantages

- Identical assay procedure
- Easy interpretation and reproducibility of results
- High diagnostic specificity and sensitivity
- Ready for automation
- Customer support

Clinical Application

BLOT-LINE ANA

- Confirmatory test for EIA ENA screen plus
- Diferential diagnosis of systemic autoimmune diseases by determination of specific ANAs

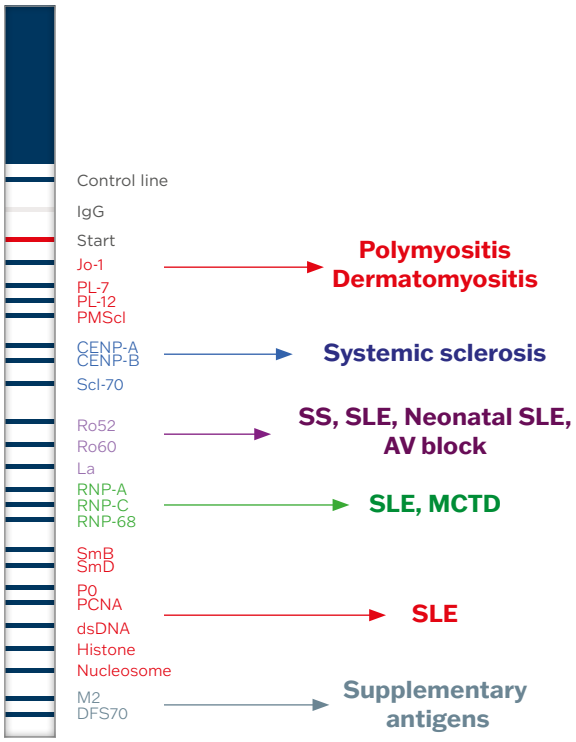
Protocol Summary

Step		Test steps
	1.	Pipette Universal solution 2 ml
	2.	Strips soaking 10 min. at room temperature – Shaker
	3.	Aspirate
	4.	Dilute samples – serum/plasma 1:51 (30 µl + 1,5 ml)
	5.	Pipette Controls and diluted samples 1.5 ml
	6.	Incubate 30 min. at room temperature – Shaker
	7.	Aspirate samples and wash strips with 1.5 ml of Universal solution 3-times for 5 min. – Shaker
	8.	Pipette Conjugate 1.5 ml
	9.	Incubate 30 min. at room temperature – Shaker
	10.	Aspirate Conjugate and wash strips with 1.5 ml of Universal solution 3-times for 5 min. – Shaker
	11.	Pipette Substrate solution (BCIP/NBT) 1.5 ml
	12.	Incubate 15 min. at room temperature – Shaker
	13.	Aspirate Substrate solution and wash strips with 2 ml of distilled water 2-times for 5 min. – Shaker
	14.	Sticking and evaluation of strips

Antigens

BLOT-LINE ANA

Recombinant antigens:
Ro52/SS-A, Ro60/SS-A, La/SS-B, RNP-A, RNP-C, RNP 68, SmB, SmD, Scl 70, Jo-1, Centromere B, Centromere A, PMScl, PL-7, PL-12, ribosomal protein P0, PCNA, dsDNA, Histones, M2, DFS70 and Nucleosome

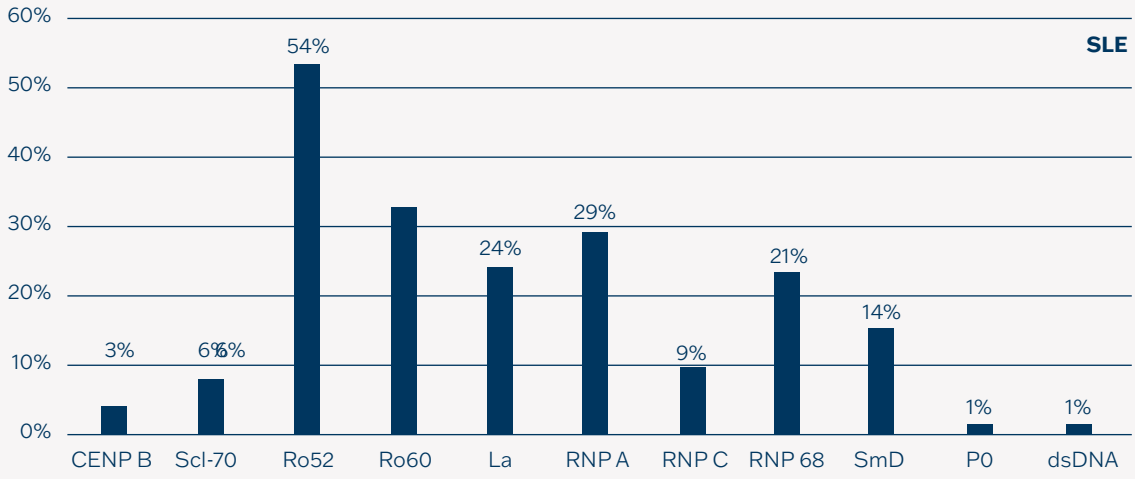


Test Characteristics

Immunoblot	Diagnostic Sensitivity	Diagnostic Specificity
BLOT-LINE ANA	97.2%	99.1%

Clinical Data

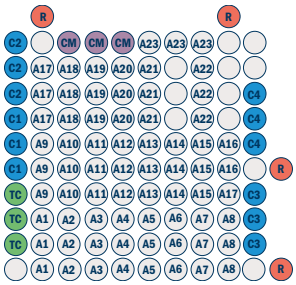
Detection of individual antigens for group of patients with SLE – results of BLOT-LINE ANA



MICROBLOT-ARRAY

Test Principle

Specific recombinant proteins (antigens) are applied to a nitrocellulose membrane, which is fitted to the well format of a microtitre plate, in the form of spots. The principle of antigen spotting is similar to that of BLOT--LINE kits. Thanks to the possibility of processing with ELISA devices, the new multiplex technology brings significant increase of efficiency in the processing of these confirmation tests.



Distribution of control spots

- **R** – Reference
- **TC** – Test control
- **CG** – Conjugate control IgG
- **C1** – Calibration 1
- **C2** – Calibration 2
- **C3** – Calibration 3
- **C4** – Calibration 4
- **A1-A44** – Antigens

User Comfort

- Low sample consumption
- Ready-to-use components
- Antigens spotted in triplicate – minimizing statistical variation
- Fully automated evaluation based on combination of positive antigen spots: quantitative (U/ml)
- Parallel identification of multiple markers
- High sensitivity
- Easy evaluation with sophisticated software
- Evaluation of the validity test through control spots

Summary Protocol

Step		Test steps
	1.	Pipette Universal solution 150 µl
	2.	Strips soaking 10 min. at room temperature
	3.	Aspirate
	4.	Dilute samples – serum/plasma 1:51 (10 µl + 500 µl) – cerebrospinal fluids 1:3 (50 µl + 100 µl) – synovial fluids 1:17,5 (10 µl + 165 µl)
	5.	Pipette Controls and diluted samples 100 µl
	6.	Incubate 30 min. at room temperature
	7.	Quick wash with Universal solution*
	8.	Aspirate samples and wash strips with 150 µl of Universal solution 3-times for 5 min.
	9.	Pipette Conjugate 100 µl
	10.	Incubate 30 min. at room temperature
	11.	Quick wash with Universal solution*
	12.	Aspirate samples and wash strips with 150 µl of Universal solution 3-times for 5 min.
	13.	Pipette Substrate solution (BCIP/NBT) 100 µl
	14.	Incubate 15 min. at room temperature
	15.	Quick wash with distilled water*
	16.	Aspirate Substrate solution and wash strips with 200 µl of distilled water 2-times for 5 min.
	17.	Dry and evaluate

*If automatic washer is used, fill the wells up to their edges and when the last well is filled, aspirate them off immediately.

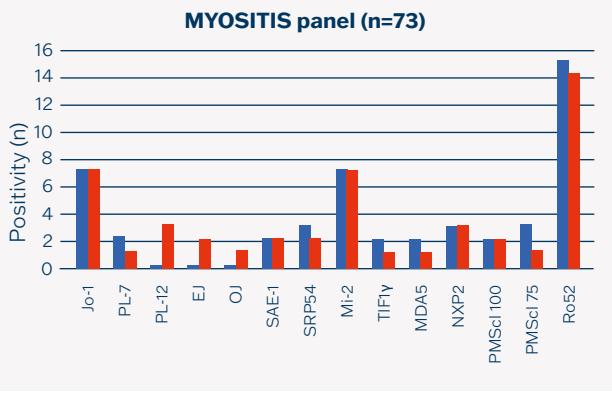
Results Processing

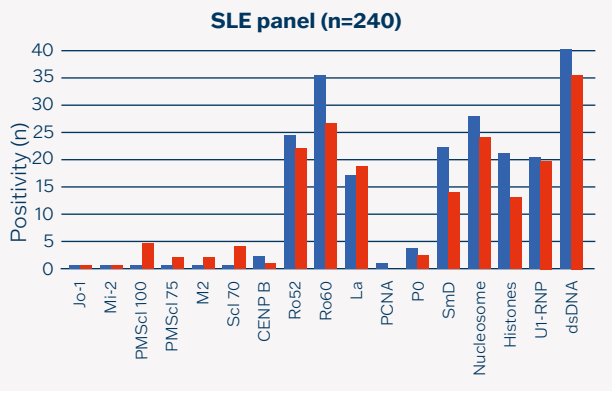
- Evaluation of individual antigens and their association with autoimmune disease type
- Possibility of evaluation of individual tests – Myopathy / SLE / Scleroderma / Overlap syndromes
- Quantitative evaluation
- Results report – cumulative/detailed

Individual antigens are grouped for evaluation purposes according to their association with the type of disease

Parameters included in the kit	Antigens
ANA	Jo-1, PL-7, PL-12, EJ, OJ, KS, YARS, ZoA, ZoB, SAE-1, SAE-2, SRP54, Mi-2, TIF1γ, MDA5, NXP2, PMScl 75, PMScl 100, CENP A, CENP B, Scl70, POLR3A, NOR90, PDGFR-β, Fibrillarin, Th/To, Ro52, Ro60, La, SmB, SmD, RNP A, RNP C, RNP 68/70, P0, Ku, Nucleolin, dsDNA, Histon, Nucleosome, PCNA | Complementary antigens: M2, DFS70.
Myositis	Jo-1, PL-7, PL-12, EJ, OJ, KS, YARS, ZoA, ZoB, SAE-1, SAE-2, SRP54, Mi-2, TIF1γ, MDA5, NXP2 | Complementary antigens: Ro52, PMScl 75, PMScl 100, Ku
Scleroderma	CENP A, CENP B, Scl70, POLR3A, NOR90, PDGFR-β, Fibrillarin, Th/To, PMScl 75, PMScl 100, RNP A, RNP C, RNP 68/70 | Complementary antigens: Ro52, Ku, M2.
SLE and other connective tissue disease	dsDNA, Histon, Nucleosome, PCNA, SmB, SmD, RNP A, RNP C, RNP 68/70, P0, Ku, Nucleolin, Ro52, Ro60, La, NOR90

Confirmatory studies





Confirmatory tests Microblot-Array

Ordering Information

ELISA

Cat. No.	Product	Units
ENA096	EIA ENA screen plus	96
ENAp12	EIA ENA profile plus	12
DNA096	EIA dsDNA	96
Jo1096	EIA Jo-1	96
Scl096	EIA Scl-70	96
Sm0096	EIA Sm	96
SSA096	EIA SS-A	96
SSB096	EIA SS-B	96
RNP096	EIA U1RNP	96
SK-ENA096	SmartEIA ENA screen plus	96
SK-ENAp12	SmartEIA ENA profile plus	12
SK-DNA096	SmartEIA dsDNA	96
SK-Jo1096	SmartEIA Jo-1	96
SK-Scl096	SmartEIA Scl-70	96
SK-Sm0096	SmartEIA Sm	96
SK-SSA096	SmartEIA SS-A	96
SK-SSB096	SmartEIA SS-B	96
SK-RNP096	SmartEIA U1RNP	96

SmartEIA kits are designed for automated processing using the Agility® analyser.

IMMUNOBLOT

Cat. No.	Product	Units
ANAL20	BLOT-LINE ANA	20

MICROBLOT-ARRAY

Cat. No.	Product	Units
ANApMA96	Microblot-Array ANA plus	96
ANAMA96	Microblot-Array ANA	96

TestLine®

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CERTIFIED ISO 9001 · ISO 13485

Company is certified to the quality management system standards ISO 9001 and ISO 13485 for in vitro diagnostics.

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