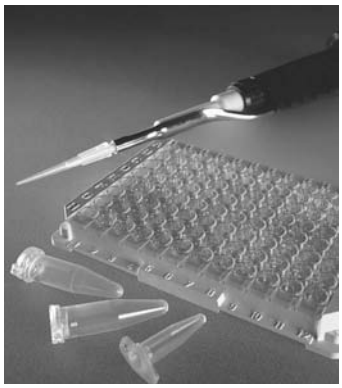
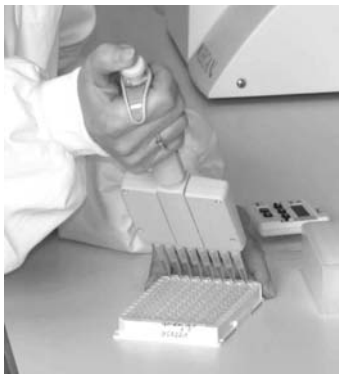
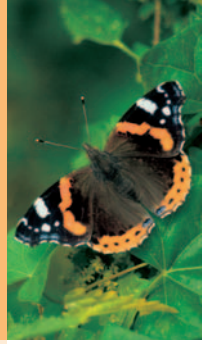
Varelisa™





ANA Profiles

EliA™
Excellence in Autoimmunity



The Varelisa™ System

Excellent clinical performance

- recombinant antigens for superb clinical specificity
- cut-offs clinically evaluated with extensive patient and disease control serum panels
- high positive predictive values for more clinically helpful results

Well established and reliable

- system introduced in 1994
- FDA cleared and CE labelled
- well represented in major international quality surveys

Automation solved

- CE-labelled package of Varelisa™assays + Dynex DSX instrument
- automation-friendly reagents
- customised solutions available

Convenient and time saving

- ready-to-use sample diluent, conjugate, substrate, calibrator and control
- one standard protocol for all assays for efficient parallel testing
- easy archiving of electronic directions for use for documentation and accreditation
- all reagents barcoded

Cost efficient and flexible

- individual patients may be assayed
- broad measuring range to minimise requirement for retesting of diluted samples

Quality is the focus

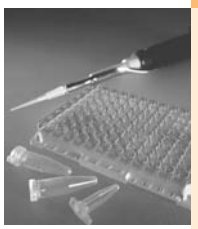
- production according to GMP guidelines
- ISO certification since 1997
- high lot-to-lot consistency due to standardised production procedures

Global network for local service and support

- serum test service
- literature service
- Pharmacia Diagnostics scientific symposia
- EliA scientific journal

The Varelisa™ ANA Profile Assays - surer diagnosis of connective tissue diseases

Semiquantitative determination of antibodies to the most clinically relevant antigens in the differential diagnosis of connective tissue diseases



High clinical relevance

- high specificity to aid in diagnostic decisions
- use of recombinant antigens minimises false positive results
- synthetic SmD peptide shows excellent specificity for SLE
- recombinant U1RNP outperforms the RNP-Sm complex in both sensitivity and specificity
- clear-cut differentiation between Sm and U1RNP
- mixture of Ro60 and Ro52 detects all relevant Ro antibodies
- high sensitivity and specificity for Scl-70, CENP-B and Jo-1 due to recombinant antigens from a eucaryotic expression system

High technical performance

- low variances and high reproducibility for consistent results
- high lot-to-lot consistency due to validated production procedures
- results expressed as a ratio to provide semi-quantitative evaluation

State-of-the-art antigens

- only recombinant antigens or synthetic peptide
- recombinant antigens produced in the baculovirus/insect cell system
- pure plasmid dsDNA
- synthetic SmD peptide carrying the SLE-specific Sm epitope

Easy handling

- ready-to-use reagents where possible
- serum or plasma can be used
- convenient incubation times
- ready for automation on microplate processors
- CE-labelled package of Varelisa™ ANA Profile assays + Dynex DSX instrument

Connective Tissue Diseases

Many of the major rheumatologic disorders are autoimmune in nature. Connective tissue diseases (CTDs) represent classical models of systemic autoimmune diseases. They are a heterogeneous group of diseases characterised by abnormal structure or function of one or more of the elements of connective tissue, i.e., collagen, elastin, or the mucopolysaccharides. Differential diagnosis of CTDs is mainly based on clinical findings but is complicated by the similarity of their symptoms. Therefore autoantibodies are useful markers to support the diagnosis of CTDs. The most prominent CTDs are systemic lupus erythematosus (SLE; potentially affecting all organs), Sjögren's syndrome (SS; characterized by diminished lacrimal and salivary gland secretion), scleroderma (systemic sclerosis, SSc; a chronic, progressive dermatosis), limited cutaneous systemic sclerosis (formerly known as CREST syndrome; with a more benign and indolent disease course), poly-/dermatomyositis (PM/DM; an acute or chronic inflammatory disease of muscle and skin), and mixed connective tissue disease (MCTD; a syndrome with features of scleroderma, rheumatoid arthritis, SLE and PM/DM). The prevalence of marker autoantibodies in particular CTDs are summarised in Table 1.

Marker autoantibody	Associated CTD	Autoantibody prevalence
dsDNA	SLE	60 - 90 %
U1RNP	MCTD, SLE	100 %, 30 - 70 %
Sm	SLE	20 - 30 %
SS-A/Ro	Sjögren's syndrome, SLE	60 - 90 %, 25 - 30 %
SS-B/La	Sjögren's syndrome, SLE	40 - 95 %, 6 - 15 %
Scl-70	systemic sclerosis	20 - 70 %
CENP	limited systemic sclerosis (CREST)	70 - 80 %
Jo-1	poly-/dermatomyositis	25 - 35 %

Table 1: *Prevalence of autoantibodies in connective tissue diseases.*

The Antigens

The use of human recombinant antigens produced in the eucaryotic baculovirus/ insect cell system and synthetic peptides guarantee good sensitivity and outstanding specificity of the Varelisa™ ANA Profile assays.

Autoantibodies can be a great help in setting up the clinical diagnosis of CTDs, however, the more disease specific they are, the more help they can provide. Therefore it's crucial for autoantibody tests to use very pure antigens in order to minimise false positive results and to reach a high specificity. Since purification of native antigens from animal cells or human cells is very difficult to achieve and often involves harsh, protein-altering processes, Pharmacia Diagnostics uses only human recombinant proteins and synthetic peptides for the Varelisa™ANA Profile assays. This avoids contaminations of the antigen which may lead to false positive results and also guarantees a high lot-to-lot consistency of the antigens. As an intact three dimensional structure of the antigens (conformation) is crucial for the recognition by antibodies to most of the ANAs, our human recombinant antigens are produced in the eucaryotic baculovirus/insect cell system, which, in contrast to bacterial systems, is capable of expressing the antigens in the correct conformation. For the Sm antigen we go even further and use a synthetic SmD peptide, carrying only the epitope of interest and thus leading to an outstanding specificity of the assay.

Again, specificity is the reason why we use pure, double-stranded, plasmid dsDNA instead of native purified DNA as our antigen thereby avoiding contamination with histones or single stranded regions, both of which lead to false positive results. The high specificity of the tests achieved with our antigens is particularly important for Profile assays as they are often used to follow-up ANA screening tests, which, depending on the method chosen, may be quite unspecific.

The Varelisa™ ANA Profile Assays

We offer three different ANA Profile assays to suit your needs. It's your choice as to which one fits best to your specific requirements.

Test	Varelisa™ ReCombi ANA Profile	Varelisa™ ReCombi ANA 4 Profile	Varelisa™ ANA Profile
Antigens included	dsDNA	U1RNP (RNP70, A, C)	RNP70
	U1RNP (RNP70, A, C)	SmD	U1RNP (RNP70, A, C)
	SmD	Ro (Ro52, Ro60)	SmD
	Ro (Ro52, Ro60)	La	Ro (Ro52, Ro60)
	La		La
	Scl-70		Scl-70
	CENP-B		CENP-B
	Jo-1		Jo-1

Table 2: *Prevalence of autoantibodies in connective tissue diseases.*

The **Varelisa™ ReCombi ANA Profile** allows a comprehensive follow-up of a positive ANA screening assay, containing all relevant markers for the most prominent connective tissue diseases.

The **Varelisa™ ReCombi ANA 4 Profile** is specialised for follow-up of speckled HEp-2 cell immunofluorescence patterns. It is also useful if you are only interested in checking U1RNP, Sm, Ro and La for other reasons, e.g. if some of the CTD entities can already be excluded by clinical presentation.

The **Varelisa™ ANA Profile** allows follow-up of a positive ANA screening assay and discrimination between antibodies directed to one or more antigens of the U1RNP mixture and RNP70, which is said to be the most specific U1RNP marker for MCTD, alone.

Connective Tissue Diseases – Differential Diagnosis

Accuracy of Results

The Varelisa™ ANA Profile assays give even more detailed and comprehensive results compared to other methods.

The Varelisa™ ANA Profile assays are calibrated against international standards or international reference preparations, respectively, wherever available. This assures a good clinical performance which is regularly checked using sera from reference panels, as e.g. the samples from the centers of Disease Control (CDC), as shown in Table 3 (example Varelisa™ ReCombi ANA Profile).

Sample	dsDNA	U1RNP	Sm	Ro	La	Scl-70	CENP	Jo-1	Target (acc. to Tan et al. (1999)
CDC 1	7.1	0.5	1.0	0.2	0.0	0.1	0.2	0.1	homogeneous/rim (dsDNA, ssDNA, Histone, weak Sm)
CDC 2	0.2	0.2	0.2	2.6	3.3	0.1	0.1	0.1	speckled (SS-B/La, weak SS-A/Ro)
CDC 3	0.2	3.4	0.2	3.0	2.8	0.1	0.1	0.1	speckled (U1RNP, Ro,La, weak Sm)
CDC 4	0.2	3.3	0.2	0.1	0.0	0.1	0.1	0.1	U1RNP
CDC 5	0.5	2.8	4.5	0.2	0.0	0.2	0.2	0.1	Sm, Histone
CDC 6	0.2	0.3	0.4	0.3	0.2	0.3	0.2	0.1	nucleolar
CDC 7	0.6	0.1	0.2	3.5	0.1	0.1	0.1	0.1	SS-A/Ro
CDC 8	0.2	0.1	0.1	0.1	0.0	0.1	4.7	0.1	Centromere
CDC 9	0.4	0.2	0.2	0.1	0.0	3.8	0.2	0.1	Scl-70
CDC 10	0.1	0.1	0.1	1.8	0.0	0.1	0.1	4.2	Jo-1

Table 3: *Performance of Varelisa™ ReCombi ANA Profile with CDC sera. CDC 5 shows an additional U1RNP reactivity which can be explained by a cross-reactivity of antibodies to SmB,B' with RNP A and C or by the higher sensitivity of the mixture of recombinant antigens compared to the RNP-Sm complex used in other assays. CDC 6 contains antibodies to nucleolar antigens, which are not included in the Varelisa™ ANA Profile assays. CDC 10 shows additional Ro activity which is due to Ro52 antibodies that are known to occur very often together with those to Jo-1 and indicate the development of secondary Sjögren's syndrome in myositis patients. However, the definition of CDC 10 did not include a check for Ro52 antibodies.*

High Clinical Relevance

In contrast to „technical" immunoassays which detect all antibodies present in a sample the Varelisa™ ANA Profile assays are developed „clinically" in order to provide help in the diagnosis of connective tissue diseases. Thus clinical relevance and specificity are their most prominent features.

Two more examples of the excellent clinical performance of the Varelisa™ ANA Profile assays are the results for Sm and U1RNP.

Sm Antibodies

Less false positive results. Positive Sm results make SLE very likely.

A recently published clinical study including 628 clinically defined samples found an excellent specificity of 99.8 % for Varelisa™ Sm, which is the same assay as the Sm part in the Varelisa™ Profiles, but allows quantitative evaluation (Mahler et al., 2004).

The superior specificity of the Varelisa™ Sm Antibodies assay compared to anti-Sm assays from several other suppliers, particularly for MCTD patients, could be demonstrated by Mahler et al. (2005) (Table 4).

Sample No.	Diagnosis	Supplier A ELISA cut-off 20 RE	Supplier B ELISA cut-off 40 Units	Supplier C Luminex cut-off 1 Ratio	Varelisa™ Sm Antibodies cut-off 13 Units
1	MCTD	87.9	118.8	1.1	3.0
2	MCTD	6.7	52.0	0.9	0.0
3	MCTD	87.4	111.7	1.5	0.5
4	MCTD	21.6	34.1	0.6	0.0
5	MCTD	10.5	41.7	0.6	1.1
6	MCTD	7.2	41.4	0.3	3.2
7	MCTD	4.2	67.1	0.5	0.7
8	MCTD	118.5	132.4	2.4	4.1
9	MCTD	12.8	42.7	0.3	2.4
10	MCTD	8.4	30.9	0.3	1.1
No.of false positives		4	8	3	0

Table 4: *Comparison of four anti-Sm assays (RE = relative units). (Taken from Mahler et al., 2005).*

RNP Antibodies

Better differentiation between Sm and RNP. Easier and surer differential diagnosis of SLE and MCTD.

All Varelisa™ Profile assays use U1RNP instead of the purified RNP-Sm complex or purified RNP proteins as antigen. U1RNP represents a mixture of the human recombinant antigens RNP70, RNP A and RNP C. Its advantage is a clear definition of the antigens and a resulting higher specificity. An internal clinical study including 36 SLE patients and 7 MCTD patients revealed that this defined antigen mixture, together with the SmD peptide antigen, provides better differentiation of antibodies to U1RNP proteins and Sm, respectively.

Easier differential diagnosis

Three out of 10 SLE samples evaluated as positive with the RNP-Sm complex had no RNP antibodies but those to Sm, which are specific for SLE (Table 5). The RNP-Sm complex gives more clinically unspecific results.

Sample No.	Diagnosis	Results in Ratio (neg. < 1.0 / equivocal 1.0 - 1.4 / pos. > 1.4) U1RNP	RNP-Sm	Sm
1	SLE	5.0	2.9	2.5
2	SLE	4.7	8.1	4.5
3	SLE	4.4	7.3	3.6
4	SLE	3.9	7.9	5.8
5	SLE	3.7	7.1	5.0
6	SLE	3.6	6.7	4.8
7	SLE	2.3	6.8	5.6
8	SLE	1.4	4.0	5.7
9	SLE	1.3	2.3	2.2
10	SLE	0.8	6.4	3.0

Table 5: *U1RNP, RNP-Sm and Sm antibodies in SLE patients. Higher specificity of recombinant U1RNP compared to the purified RNP-Sm complex.*

Better sensitivity

The recombinant U1RNP detected two more MCTD patients than the RNP-Sm complex (Table 6).

Sample No.	Diagnosis	Results in Ratio (neg. < 1.0 / equivocal 1.0 - 1.4 / pos. > 1.4) RNP70	U1RNP	RNP-Sm	Sm
11	MCTD	3.7	3.8	5.5	0.2
12	MCTD	2.8	3.8	6.6	1.1
13	MCTD	3.7	3.8	3.3	0.1
14	MCTD	3.5	3.8	3.5	0.4
15	MCTD	3.5	3.8	5.0	0.2
16	MCTD	3.0	3.6	0.9	0.1
17	MCTD	3.0	3.1	0.5	0.1

Table 6: *RNP70, U1RNP, RNP-Sm and Sm antibodies in MCTD patients. Higher sensitivity of recombinant U1RNP compared to the purified RNP-Sm complex.*

Ro Antibodies

Mixture of Ro52 and Ro60 lead to better sensitivity for Sjögren's syndrome.

The SS-A/Ro antigen is a good example for the Varelisa™ ANA Profile assays providing not only high specificity but also high sensitivity. The antigen consists of a mixture of both human recombinant Ro60 and Ro52. Many other ELISA assays only contain Ro60 and thus do not detect patients having only Ro52 antibodies. However, these antibodies are important markers for both Sjögren's syndrome and the risk of congenital heart block of the newborn child when present in pregnant women (Salomonsson et al., 2002). Their importance for Sjögren's syndrome was recently confirmed by Garberg et al. (2005) who showed that, depending on the clinical criteria for diagnosis used, 15 - 20 % of Sjögren's syndrome patients had only antibodies to Ro52 and not to Ro60 or La (Table 7). Assays which do not include Ro52 would miss these patients.

Serum autoantibodies	Preliminary european classification criteria of 1993 (Vitali et al., 1993) n = 100 No. of Sjögren's syndrome patients in %	Revised european classification criteria of 2002 (Vitali et al., 2002) n = 66 No. of Sjögren's syndrome patients in %
only Ro52	20	15
total Ro52	62	71
only Ro60	0	0
total Ro60	24	33
only La48	4	3
total La48	45	56
Ro52 + La48	20	24
Ro60 + La48	2	2
Ro52 + Ro60	3	5
Ro52 + Ro60 + La48	19	27

Table 7: *Prevalence of Ro52, Ro60 and La48 antibodies in Sjögren's syndrome patients. European classification criteria of 1993 (Taken from Garberg et al., 2005).*

dsDNA Antibodies

The use of plasmid dsDNA results in high sensitivity and specificity.

One critical antigen in terms of clinical relevance is dsDNA. Since only antibodies to pure double stranded dsDNA are specific for SLE, we use a circular plasmid dsDNA in our Varelisa™ ReCombi ANA Profile assay. Therewith both contamination with histones or histone-like proteins and single-stranded regions, which may both lead to false positive results, are avoided. The high values for both sensitivity and specificity of the Varelisa™ dsDNA Antibodies have been proven by independent publications (e.g. Tan et al., 1999; Fig. 1).

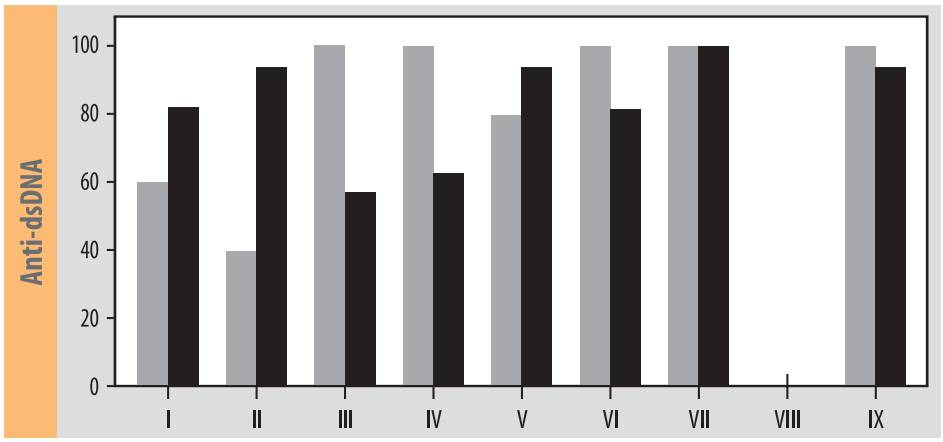


Figure 1: *Sensitivity and specificity of the Varelisa™ dsDNA Antibodies assay (Kit VII) compared to 7 anti-dsDNA assays of other suppliers. Gray bars denote percent sensitivity and black bars percent specificity. (Taken from Tan et al., 1999).*

La, Scl-70, CENP-B, Jo-1 antibodies

The remaining antigens, La, Scl-70, CENP-B and Jo-1, are produced using the same principles and methods in order to provide the same excellent clinical performance as shown in the examples above.

Summary

The Varelisa™ ANA Profile assays show an excellent clinical performance and make the differential diagnosis of connective tissue diseases easier and surer.

References:

- Mahler M, Fritzler MJ, Blüthner, M (2004): Identification of a SmD3 epitope with a single symmetrical dimethylation of an arginine residue as a specific target of a subpopulation of anti-Sm antibodies. *Arthritis Res Ther 7: R19 - R29*
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We know how

PHARMACIADiagnostics – the recognised worldwide leader in allergy, asthma and autoimmunity testing

All allergy and asthma activities are coordinated in Uppsala, Sweden, while Freiburg is the Centre of Excellence for the development, production and marketing of diagnostic tests for autoimmune diseases. To meet our commitment in autoimmune diagnostics we invested in a new Biotechnikum II for research, development and production.

Our scientific competence – your advantage

Diagnostic tests for the detection of autoantibodies are based on the specific antigen-antibody binding reaction. Some of the antigens are only found in very small quantities in human cells and hence are very hard to isolate by conventional methods. An optimal alternative is the use of recombinant human antigens which can be produced in the required amounts by methods which allow more gentle purification with reduced risk of contamination. Pharmacia Diagnostics uses the Baculovirus/insect cell expression system for the production of most of their antigens. These recombinant antigens have a conformation corresponding to their native counterparts in human tissue.
This ensures highest antigenicity of the proteins and guarantees assays of an outstanding sensitivity and specificity.

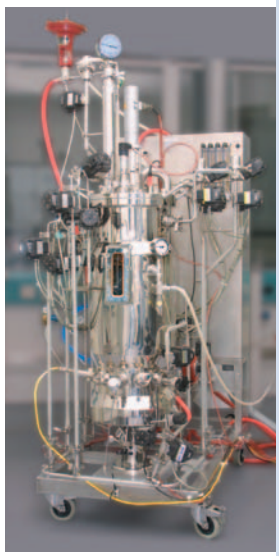
Our services – your support

You can rely on Pharmacia Diagnostics' commitment to provide you with the range of services and support you require in your daily clinical and laboratory routines.

We offer you:

- Scientific EliA Journal
- Serum test service
- Technical assistance / consultation
- Literature service
- Customer workshops
- Scientific cooperations
- Products manifactured according to GMP and ISO 9001, 46001
- Participation in quality assessment programs:
  - NEQAS (U.K.)
  - INSTAND (Germany)
  - CAP survey (U.S.)

Large-scale purification according to GMP guidelines leads to long-term reproducibility



Excellent antigen quality through controlled and automated fermentation

High consistency through automated coating and large batch sizes

Technical Data

Product		Varelisa™ ReCombi ANA Profile Varelisa™ ReCombi ANA 4 Profile Varelisa™ ANA Profile
Antigens		circular plasmid dsDNA from E. coli human recombinant RNP70 human recombinant U1RNP (RNP70, A, C) synthetic SmD peptide human recombinant Ro (Ro52, Ro60) human recombinant La human recombinant Scl-70 human recombinant CENP-B human recombinant Jo-1
Standardisation		one calibrator for semiquantitative evaluation (ratio)
Cut-off		neg. < 1.0 ratio; pos. > 1.4 ratio
Dilution		1:101
Sample material		Serum, Plasma (EDTA, citrate)
Reproducibility	Intra-assay variance Inter-assay variance	1.8 % - 7.9 % 1.0 % - 7.2 %

Ordering Information

	Package size	*Article No.*
Varelisa™ ReCombi ANA Profile	96 determinations / 10 patient profiles	18496
Varelisa™ ReCombi ANA 4 Profile	96 determinations / 22 patient profiles	18596
Varelisa™ ANA Profile	96 determinations / 10 patient profiles	18396

PHARMACIADiagnostics

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