AI-Line: A flexible and reliable product line for the detection of autoantibodies

Background

Autoantibodies (aabs) contribute significantly to the diagnosis of systemic autoimmune rheumatic diseases (SARD). The AI-Line represents an ELISA product line for the semi-quantitative detection of aabs in human serum or plasma. All antigens used in the ELISAs are carefully chosen, comprising highly purified native or recombinant proteins or synthetic peptides. The AI-Line ELISAs were developed in close collaboration with world leading experts and research groups specialized in the diagnosis of SARD. Based upon this close collaboration, all AI-Line ELISAs were individually optimized for highest diagnostic sensitivity and specificity. This is reflected in the excellent diagnostic performance, demonstrated in a lot of international clinical studies. In addition to the excellent diagnostic performance the AI-Line ELISAs offer a very user-friendly and easy-to-automate handling procedure. All tests use the same Sample and Washing Buffer and the same incubation-time and scheme, which gives the user the possibility to process flexible and patient specific test routines either manually or with an automated platform (see figure 1). All tests are based on one point calibration and contain a positive and a negative control.

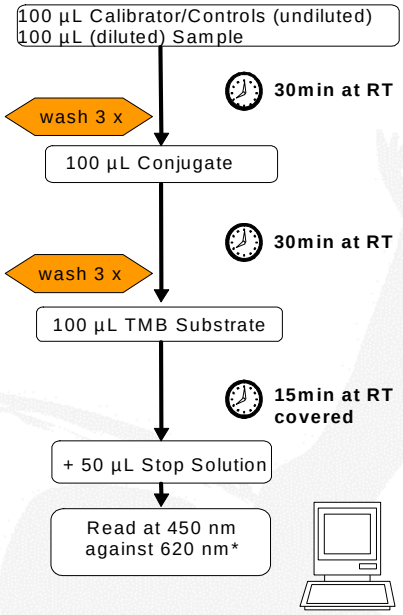

Figure 1 Test scheme AI-Line ELISA.

ANA Screen (REF: 25012)

The ANA Sceen ELISA is designed as a first line screening test and detects the clinically most important aab against double stranded DNA (dsDNA), Ro (SS-A), La (SS-B), Smith Antigen (Sm), ribonukleoproteins (RNP), topoisomerase I (Topo I, Scl-70), centromere (CENP), $tRNA_{His}$ synthetase (Jo-1) and PM1-Alpha (PM/Scl). Each aab has a different clinical sensitivity, specificity and disease association (see table 1).

Table 1 Antigens and associated diseases for AI-ELISAs

Antigen	Association
dsDNA (r)	Systemic Lupus Erythematosus (SLE)
Ro52 (r)	SLE, SjS, SSc
Ro60 (r)	Sjögren Syndrom (SjS)
La (r)	Sjögren Syndrom (SjS)
RNP/Sm (n)	MCTD
Sm (n)	SLE
Jo-1 (r)	Polymyositis (PM)
Scl-70 (r)	Systemic Sclerosis (SSc)
CENP (r)	Systemic Sclerosis (SSc)
PM1-Alpha (s)	PM/SSc overlap syndrome
Rib-P(S)	SLE

The main advantage of the ANA Screen ELISA compared to indirect immunofluorescence (IIF) is the simple and easy-to-automate handling and the fast interpretation of the results. It is undoubtable that the ANA Screen ELISA cannot compete with the antigen diversity of HEp-2 cells but the most relevant antigens are contained in the ELISA. Autoantibodies targeting other antigens than those contained in the test are rarely found, and often coincide with aabs against the main autoantigens presented in the ANA Screen ELISA. Therefore, even sera with rarely occurring aabs are often found positive in the ANA Screen ELISA. Furthermore, some antigens presented in HEp2 cells are of limited diagnostic value and clinical relevance leading to false positive results, such as the DFS70 protein (***d**ense **f**ine **s**peckled* Antigen 70 kDa) [1]. Autoantibodies directed against DFS70 are not associated with SARD. Of note, some aabs of high clinical relevance (e.g. anti-Jo-1, anti-Ro52, anti-Ro60, anti-Rib-P) can only be detected with low sensitivity by IIF [2]. The ANA Screen ELISA shows excellent correlation with confirmation tests such as the single parameter ELISAs of the AI-Line or *recom*LINE ANA/ENA Line-immuno-assays (Mikrogen). This has recently been shown as poster presentation on the 10th Congress on autoantibodies in Guadalajara, Mexico 2008 [3]. To further improve the reliability of ANA Screen ELISA tests, a novel coating strategy has been developed, that allows for a high coating density of dsDNA beside the protein antigens, resulting in an outstanding sensitivity of the ANA Screen ELISA for dsDNA antibodies.

PM1-Alpha (REF: 25001)
The PM1-Alpha ELISA represents a special highlight of the AI-Line. Due to the use of a highly purified and highly immunogen synthetic peptide this test reaches a significantly higher sensitivity and specificity than common methods for the detection of anti-PM/Scl aabs [4,5]. In a recent study performed by a leading expert, Prof. Humbel (Centre Hospitalier, Luxembourg), the superiority of the PM1-Alpha ELISA to recombinant antigens such as PM/Scl-75c and PM/Scl-100 was demonstrated [6].

SS-A (Ro60), Ro52 and SS-B (REF: 25006-8)
For the detection of anti-SS-A (Ro60), anti-Ro52 and anti-SS-B aabs the AI-Line offers the single parameter ELISA tests Ro52, Ro60 and La. The SS-A (Ro60) ELISA is the first commercially available ELISA which uses a brand new recombinant Ro60 protein offering highly consistent results without contaminating proteins often found with native purified antigens. The separate detection of anti-Ro52 and anti-Ro60 aabs is of great importance, because single reactivity to either Ro52 or SS-A (Ro60) can be missed when measured with a classical SS-A ELISA based on a mixture of both antigens. Around 20% of anti-Ro52 or SS-A (Ro60) positive may remain undetected using a mixture of both antigens [7,8].

Ribosomal P (REF: 25002)
The detection of anti-Rib aabs is of great clinical relevance because of the high specificity for SLE [9]. The sensitivity of IIF for the detection of anti-Rib-P aabs is poor; in IIF far more than 50% of anti-Rib-P positive samples do not show the typical cytoplasmatic fluorescence staining pattern. Therefore patients suspected to suffer from SLE should be tested for anti-

Rib-P aabs by ELISA. The careful choice of the antigen used in the assay is also of great importance because aabs against the ribosomal P2 protein also can be found in sera of patients suffering from a Hepatitis C viral infection (HCV) with no clinical evidence of SLE. Therefore, the Rib-P ELISA was designed based on a highly pure synthetic peptide containing only the SLE specific epitope.

dsDNA (REF: 25005)
The detection of anti-dsDNA aabs can be done using a variety of methods. Next to the classical IIF test using *Crithidia lucillae* cells as substrate (CLIFT), dsDNA ELISA tests are widely used. Though the classical CLIFT has a high specificity it only offers a poor sensitivity [10]. The AI-Line dsDNA ELISA is based on a highly purified recombinant plasmid DNA and combines high sensitivity and specificity for SLE and thus allows a reliable SLE-diagnosis [11].

RNP/Sm (REF: 25010; 25011)
The AI-Line offers two assays for the detection of RNP/Sm antibodies. The RNP/Sm ELISA test uses a highly purified native antigen, which contains all RNP/Sm antigens as a complex and allows for a highly sensitive detection of anti-RNP/Sm antibodies. The Sm ELISA is based on a pure native SmD antigen. While anti-RNP/Sm aabs can be found in different SARD, aabs against the SmD autoantigen are highly specific for SLE [11]. Both the RNP/Sm and the Sm ELISA show very good agreement with reference methods for the detection of anti-RNP/Sm antibodies. As SmD proteins contain the modified amino acid dimethylarginine (DMA) which cannot be produced in a recombinant format, native antigens or synthetic peptides should be preferred for the detection of anti-Sm antibodies.

Scl-70 and CENP B (REF: 25003;25004)
The AI-Line offers two assays for the detection of systemic sclerosis (SSc) specific antibodies based on recombinant proteins expressed in insect cell culture. Anti-Scl-70 aabs were historically believed to be highly specific for SSc. In recent years anti-Scl-70 were found in sera of patients suffering from SLE, and anti-CENP-aabs in sera of patients suffering from primary biliary cirrhosis. In a recent study performed with the AI-Line Scl-70 ELISA the known high disease specificity for SSc could be confirmed [12].

Jo-1 (REF 25009)
The antigen Jo-1 (Aminoacyl-tRNA Synthetase) was first described in the serum of a myositis patient. A large number of patients with anti-synthetase syndrome suffer from interstitial lung disease, Raynaud's Phenomenon, mechanic's hands and arthritis. Anti-Jo-1 aabs are strongly associated with isolated anti-Ro52 aabs [8]. More than 70% of all anti-Jo-1 positive sera in myositis also show anti-Ro52 reactivity. Apart from Jo-1, other tRNA synthetases (e.g. PL-7, PL-12, OJ) can be the target of aabs, but with lower frequency.

Disease specific profile ELIAs (REF: 25019, 25021; 25023)
For symptome-oriented aab-testing the AI-Line offers disease specific profile-ELISAs containing the clinically relevant antigens on user friendly colour coded microtiter stripes. Available are:
-SLE Profile 4 (Ref: 25019) offering antigens dsDNA, Sm, RNP-Sm and Rib-P
-SLE Profile 6 (Ref: 25021) offering antigens dsDNA, Sm, RNP-Sm, Rib-P, Ro52 and La-SSB

-ENA Profile 8 (Ref: 25023) offering antigens dsDNA, RNP-Sm, Sm, Ro52, La-SSB, Scl 70, CENP and Jo-1.

CENP A ELISA (Ref: 25024)

The CENP-A ELISA is the first assay for the detection of ACA using a novel CENP-A derived peptide (patent pending) showing significantly higher specificity compared to other assays. Anti-centromere antibodies are found in 20-50% of patients with SSc depending on the detection system and on the makeup of the patient cohort, but can also be observed in other disease conditions such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). Epitope-mapping studies revealed that CENP-A and CENP-B are the major centromere autoantigen. Several studies showed an association between anti-CENP antibodies and the CREST syndrome (*calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia*).

Literature

1. Watanabe A, Kodera M, Sugiura K, Usuda T, Tan EM, Takasaki Y *et al.*: **Anti-DFS70 antibodies in 597 healthy hospital workers.** *Arthritis Rheum* 2004, **50:** 892-900.

2. Hoffman IE, Peene I, Veys EM, de Keyser F: **Detection of specific antinuclear reactivities in patients with negative anti-nuclear antibody immunofluorescence screening tests.** *Clin Chem* 2002, **48:** 2171-2176.

3. Mahler M, Eisfeller P, Silvermann ED, Fritzler MJ: **Diagnostic value of a novel ANA Screen ELISA.** *10th International Workshop on autoantibodies and autoimmunity, Guadalajara* 2008.

4. Mahler M, Raijmakers R, Dahnrich C, Bluthner M, Fritzler MJ: **Clinical evaluation of autoantibodies to a novel PM/Scl peptide antigen.** *Arthritis Res Ther* 2005, **7:** R704-R713.

5. Mahler M, Raijmakers R: **Novel aspects of autoantibodies to the PM/Scl complex: clinical, genetic and diagnostic insights.** *Autoimmun Rev* 2007, **6:** 432-437.

6. Mahler M, Fritzler MJ: **PM1-Alpha ELISA: the assay of choice for the detection of anti-PM/Scl autoantibodies?** *Autoimmun Rev* 2009, **8:** 373-378.

7. **Ro/SS-A Antibodies.** In *Autoantibodies in Systemic Autoimmune Diseases*. Edited by Conrad K, Schoessler W, Hiepe F, Fritzler MJ. Lengerich: Pabst Science Publishers; 2008:167-172.

8. Schulte-Pelkum J, Fritzler M, Mahler M: **Latest update on the Ro/SS-A autoantibody system.** *Autoimmun Rev* 2009, **8:** 632-637.

9. Mahler M, Kessenbrock K, Szmyrka M, Takasaki Y, Garcia-De La Torre I, Shoenfeld Y *et al.*: **International multicenter evaluation of autoantibodies to ribosomal P proteins.** *Clin Vaccine Immunol* 2006, **13:** 77-83.

10. Mahler M, Fritzler MJ: **Anti-dsDNA antibody testing in the clinic: Farr or ELISA?** *Nat Clin Pract Rheumatol* 2007, **3:** 72-73.

11. Mahler M, Fritzler MJ, Bluthner M: **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* 2005, **7:** R19-R29.

12. Mahler M, Petschinka M, Luettich T, Hiepe F, Szmyrka-Kaczmarek M, Fritzler M: **Anti-Topo-I/Scl-70 Autoantibodies in systemic Lupus Erythematosus: myth or reality?** *6th International Congress on Autoimmunity, Porto* 2008, Poster Presentation.