FACULTY OF MEDICINE | UNIVERSITY OF CALGARY

DR. FOOKE LABORATORIEN GmbH

A Comparison Between Old and Newer Methods for the Detection of Anti-Ribosomal P Autoantibodies

Ngo, Jennifer[1], Mahler, Michael[2] and Fritzler, Marvin J[1]

[1] Faculty of Medicine, University of Calgary, Calgary, AB, Canada. [2] Dr. Fooke Laboratorien GmbH, Neuss, Germany.

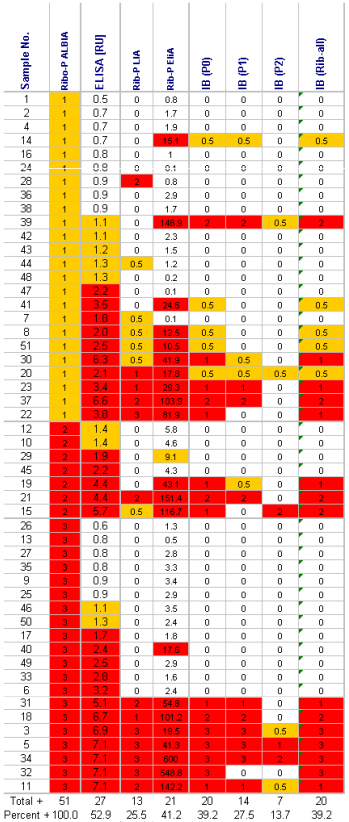
Background: Anti-ribosomal P (Rib-P) autoantibodies (aab) represent a highly specific serological marker for the diagnosis of systemic lupus erythematosus (SLE). The antigen targets of anti-Rib P aab are three ribosomal P proteins (P0, P1 and P2). Known since more than 25 years, anti-Rib-P aab have not achieved the attention or clinical utility that anti-Sm or anti-dsDNA aab have. This might be attributed to the limited reliability of indirect immunofluorescence (IIF) assays for the detection of these aab, the lack of access to international reference sera and misunderstanding of the clinical relevance of anti-Rib-P aab. A variety of methods are currently available for the detection of anti-Rib-P aab. The objective of this study was to compare well established methods with newer technologies for the detection of anti-Rib-P aab.

Methods: Sera (n=51) with putative anti-Rib-P reactivity were identified by an addressable laser bead assay (ALBIA: INOVA) and tested for anti-Rib-P aab different methods (Table 1).

Table 1. Assays used for the detection of anti-Rib-P antibodies

Assay	Product / Manufacturer	Antigen(s)	Cut-off
Line immunoassay (LIA)	recomLine ANA/ENA / Mikrogen, Germany	Recombinant P0	Band Rib-P > control band
Indirect immunofuorescence (IIF)	ANA test kit / ImmunoConcepts, US	Native ribosomes in HEp-2 cells	CSP > 1:80
Immunoblot (IB)	In-house assay / Phadia, Germany	Native P0, P1 and P2	Clearly visible lines
Addressable laser bead assay (ALBIA)	QUANTA Plex™ SLE profile 8 / Inova, US	Synthetic peptide	0 = negative 1 = weakly positive 2 = positive 3 = strongly positive
ELISA	Ribosomal P ELISA / Dr. Fooke, Germany	Synthetic peptide	< 1 RU negative 1 – 1.5 RU borderline > 1.5 RU positive
EliA® Rib-P	EliA® Rib-P / Phadia, Germany	Recombinant P0, P1 and P2	7-10 units borderline; > 10 positive

Results and findings: Depending on the assay, 14-53% of the sera selected on the basis of a positive Rib-P as detected by ALBIA were positive in another assay (Fig 1). When the IB results were used as reference excellent to moderate discrimination between Rib-P aab was observed (Fig 2 a.). Agreement to IIF was significantly lower (Fig 2 b.). When individual Rib-P components were evaluated, the frequency was P0/P1/P2> P0 alone> P1 alone> P2 alone. Good quantitative agreement was observed between ELISA and EliA® (Fig 3). Anti-Rib-P aab were associated with other aab, most notably with anti-Ro60 (Table 1).



Sample No.	Rib-P ALBIA	ELISA [RU]	Rib-P LIA	Rib-P EliA	IB (P0)	IB (P1)	IB (P2)	IB (Rib.-all)
1	1	0.5	0	0.8	0	0	0	0
2	1	0.7	0	1.7	0	0	0	0
4	1	0.7	0	1.9	0	0	0	0
14	1	0.7	0	15.1	0.5	0.5	0	0.5
16	1	0.8	0	1	0	0	0	0
24	1	0.9	0	0.1	0	0	0	0
28	1	0.9	2	0.8	0	0	0	0
36	1	0.9	0	2.9	0	0	0	0
38	1	0.9	0	1.7	0	0	0	0
39	1	1.1	0	146.9	2	2	0.5	2
42	1	1.1	0	2.3	0	0	0	0
43	1	1.2	0	1.5	0	0	0	0
44	1	1.3	0.5	1.2	0	0	0	0
48	1	1.3	0	0.2	0	0	0	0
47	1	2.2	0	0.1	0	0	0	0
41	1	3.5	0	24.6	0.5	0	0	0.5
7	1	1.8	0.5	0.1	0	0	0	0
8	1	2.0	0.5	12.5	0.5	0	0	0.5
51	1	2.5	0.5	10.5	0.5	0	0	0.5
30	1	6.3	0.5	41.9	1	0.5	0	1
20	1	2.1	1	17.8	0.5	0.5	0.5	0.5
23	1	3.4	1	29.3	1	1	0	1
37	1	6.6	2	103.9	2	2	0	2
22	1	3.8	3	81.9	1	0	0	1
12	2	1.4	0	5.8	0	0	0	0
10	2	1.4	0	4.6	0	0	0	0
29	2	1.9	0	9.1	0	0	0	0
45	2	2.2	0	4.3	0	0	0	0
19	2	4.4	0	43.1	1	0.5	0	1
21	2	4.4	2	151.4	2	2	0	2
15	2	5.7	0.5	116.7	1	0	2	2
26	3	0.6	0	1.3	0	0	0	0
13	3	0.8	0	0.5	0	0	0	0
27	3	0.8	0	2.8	0	0	0	0
35	3	0.8	0	3.3	0	0	0	0
9	3	0.9	0	3.4	0	0	0	0
25	3	0.9	0	2.9	0	0	0	0
46	3	1.1	0	3.5	0	0	0	0
50	3	1.3	0	2.4	0	0	0	0
17	3	1.7	0	1.8	0	0	0	0
40	3	2.4	0	17.5	0	0	0	0
49	3	2.5	0	2.9	0	0	0	0
33	3	2.8	0	1.6	0	0	0	0
6	3	3.2	0	2.4	0	0	0	0
31	3	5.1	2	54.2	1	1	0	1
18	3	6.7	1	101.2	2	2	0	2
3	3	6.9	3	19.5	3	3	0.5	3
5	3	7.1	3	41.3	3	3	1	3
34	3	7.1	3	600	3	3	2	3
32	3	7.1	3	548.8	3	0	0	3
11	3	7.1	2	140.2	1	1	0.5	1
Total +	51	27	13	21	20	14	7	20
Percent +	100.0	52.9	25.5	41.2	39.2	27.5	13.7	39.2

Figure 1 Ribosomal P reactivity profile of 51 Rib-P ALBIA positive sera detected by LIA, ELISA, EliA® immuno-blot (IB: P0, P1, P2). Results were clustered accroding to the order: ALBIA results, ELISA and LIA.

Table 1. Association of anti-Rib-P and other autoantibodies

Autoantibody	*ALBIA*	LIA
Anti-Ro60	*p*=0.0003	*p*=0.0002
Anti-Ro52	*p*=0.0218	*p*=0.0622
Anti-Sm	*p*=0.0008	n.a.
Anti-SmD	n.a.	*p*=0.0088
Anti-dsDNA	n.a.	*p*=0.077

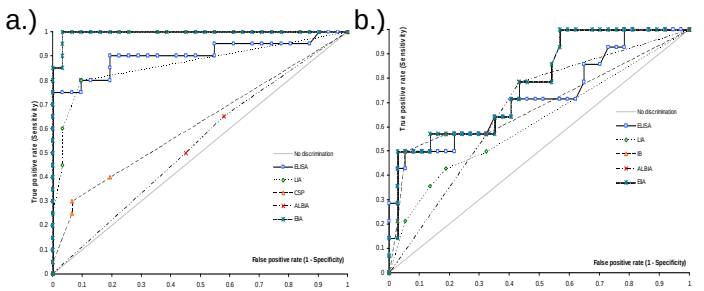


Figure 2 Receiver operating characteristics (ROC) analysis. Different methods for the detection of anti-Rib-P antibodies were compared to the results of the Immunoblot (IB) a.) and indirect immunofluorescence (IIF). Area under the curve (AUC) values were calculated as follows: 0.90 (ELISA), 0.87 (LIA), 0.62 (IIF as CSP) and 1.0 (EliA®). For IIF as reference the AUC were: 0.72 (ELISA), 0.62 (LIA), 0.69 (IB), 0.78 (EliA®).

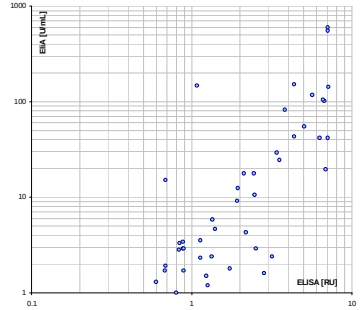


Figure 3 Correlation between ELISA and EliA®. Good quantitative agreement was observed between ELISA and EliA® (r = 0.71, 95% Confidence interval 0.54 to 0.83; p < 0.0001) according to Spearman.

Conclusions: Based on our findings, we conclude that the degree of agreement between well established and novel methods for the detection of anti-Rib-P aab vary significantly depending on the assay. Together with our previous data showing that IIF has limited reliability for the detection of anti-Rib-P aab the results of the present study reveal that IIF and IB might by replaced by novel technologies for the detection of anti-Rib-P aab.

Mahler, Ngo & Fritzler. Arthritis Res Ther 10: R31, 2008.

Mitogen Advanced Diagnostics Laboratory, University of Calgary, 3330 Hospital Dr NW, Calgary, AB, Canada T2N 4N1 (403)-220-4582 Internet: www.mitogen.ca – email: madl@ucalgary.ca

Dr. Fooke Laboratorien GmbH – Mainstraße 85 – 41469 Neuss – Germany Phone: +49 2137 1005-0 – Fax: +49 2137 12409 – Internet: www.fooke-labs.de – email: information@fooke-labs.de