

Technical and Clinical Evaluation of Anti-ribosomal P Protein Immunoassays

M. Mahler,^{1*} K. Kessenbrock,² J. Raats,^{3,5} and M.J. Fritzler⁴

¹Pharmacia Diagnostics, Freiburg, Germany

²Institute of Molecular Genetics, University of Heidelberg, Heidelberg, Germany

³Department of Biochemistry, University of Nijmegen, Nijmegen, The Netherlands

⁴Faculty of Medicine, University of Calgary, Calgary, Canada

⁵ModiQuest B.V., Nijmegen, The Netherlands

Autoantibodies to the three ribosomal phospho (-P) proteins P0, P1, P2, referred to as Rib-P, are specifically found in 10–40% of patients with systemic lupus erythematosus. The variations in the observed frequency of these autoantibodies is related to a number of factors such as the test system used to detect the antibodies. Several immunoassays that were designed for research and diagnostic laboratory use have been developed. The autoantigens employed in these tests include native proteins, recombinant polypeptides, and synthetic peptides. In this study, we compared the technical and clinical accuracy of anti-Rib-P antibody assays from different commercial suppliers including ELISA systems and a novel addressable laser bead

assay (from Euroimmun, MBL, Pharmacia Diagnostics, INOVA). Although the assays from all suppliers used in this study performed well in the technical part of the study, relatively poor correlations and significant differences in the clinical accuracy were found. Based on the results, we conclude that the detection of anti-Rib-P antibodies strongly depends on both the nature of the antigen and the detection system. We recommend that anti-Rib-P assays should be standardized on an international level. The Varelisa[®] Rib-P profile and the addressable laser bead Rib-P assays represent promising tools and platforms for the detection of anti-Rib-P antibodies in the future. *J. Clin. Lab. Anal.* 18:215–223, 2004. © 2004 Wiley-Liss, Inc.

Key words: autoantibody; SLE; Luminex; Rib-P; ELISA, ribosome

INTRODUCTION

Autoantibodies to the three ribosomal phospho (-P) proteins P0, P1, P2, referred to as Rib-P, are specifically found in patients with systemic lupus erythematosus (SLE) (1–3). The Rib-P antigen consists of three protein components of the 60S ribosomal subunit designated P0(38 kDa), P1(19 kDa), and P2(17 kDa). A pentameric complex of one copy of P0 and two copies each of P1 and P2 interacts with the 28S rRNA molecule to form a GTPase domain that is active during the elongation step of protein translation (2). The major immunoreactive epitope of these ribosomal antigens has been localized to the carboxy terminal domain, which is present in all three proteins and contains two phosphorylated serine residues (e.g., Ser¹⁰² and Ser¹⁰⁵ of human P2) (1,4,5,6). Several studies have shown that both the acidic and hydrophobic clusters, but not the phosphorylation of the P proteins, are critical for antibody binding (4,5).

Furthermore, epitope mapping studies have shown that the major epitope is located within the last six amino acids (GFGLFD) (5).

The reported prevalence of anti-Rib-P antibodies in SLE ranges from 10–40%. The prevalence is increased in Asian patients relative to the prevalence in black and Caucasian patients (7–9). The variation in the observed frequency is related to a number of factors but is, in large part, dependent on the test system used to detect the antibodies. An immunoblot technique was reported to be the most sensitive (10). Several ELISA systems

Grant sponsor: Canadian Institutes of Health Research; Grant number: MOP-38034.

*Correspondence to: Dr. Michael Mahler, Dr. Fooke Laboratorien GmbH, Mainstr. 85, 41469 Neuss, Germany.
E-mail: m.mahler.job@web.de

Received 17 September 2003; Accepted 15 October 2003

DOI 10.1002/jcla.20026

Published online in Wiley InterScience (www.interscience.wiley.com).

designed for research studies as well as diagnostic laboratory use have been developed (reviewed in 3). The antigenic analytes employed in these tests included purified native proteins, recombinant polypeptides, a synthetic peptide comprising the 22 C-terminal amino acids (C22), or a multiple antigen peptide (MAP) construct (1,9,10,11–14).

Anti-Rib-P antibodies are mainly detected in patients during the active phase of SLE (15,16) and are believed to be correlated with lupus nephritis (16,17) or hepatitis (18). The association of anti-Rib-P with central nervous system involvement and neuropsychiatric manifestations in SLE has been more controversial (1,3,5,19,32). Moreover, it became evident that anti-Rib-P antibodies are more prevalent in juvenile-onset SLE than in adult-onset SLE (20).

Although several studies have analyzed the accuracy of anti-Rib-P research assays, no data is available that allows a comparison of various anti-Rib-P assays for diagnostic laboratory use (1,5,9,10,11–14). Therefore, this study was designed to evaluate the accuracy of anti-ribosomal antibody tests from different suppliers, including conventional ELISA systems and a novel addressable laser bead assay. The first part of the study is focused on the technical sensitivity of the different commercial products and research kits. In the second part, the clinical accuracy of all these kits is investigated.

MATERIALS AND METHODS

SERUM SAMPLES

STUDY I—TECHNICAL SENSITIVITY

Anti-Rib-P-sera ($n = 30$) were selected based on the reactivity in immunoblots performed with HeLa cell cytosol extracts as described below (see Immunoblotting With Cytosol Extract). Two negative controls were selected that showed no reactivity with the ribosomal P proteins in immunoblot.

STUDY II—CLINICAL ACCURACY

Sera were collected from systemic lupus erythematosus (SLE; $n = 50$) and various control diseases including rheumatoid arthritis (RA, $n = 50$), mixed connective tissue disease (MCTD, $n = 17$), scleroderma (SSc, $n = 17$), polymyositis/dermatomyositis overlap syndrome (PM/DM, $n = 11$), and other autoimmune disorders ($n = 15$). All patients were classified according to published criteria for each disease (21–25). Sera were stored at -80°C until use.

ANTI-RIB-P ASSAYS

Research kits

Varelista[®] C22, P0, P1, and P2. Four independent research ELISA systems were recently developed (5). The cutoff value was defined at 6.1 U/mL for each assay.

Varelista[®] rib-P profile. Microtiter plates were coated with the C22 peptide and with the recombinant Rib-P proteins P0, P1, and P2, each of the antigens in separate wells. A calibrator was developed using an anti-Rib-P serum and adjusted to an optical density of around 1.000 mOD. The cutoff value on OD level was determined for each antigen separately after the testing of various controls ($n = 100$). A conversion factor (CF) was calculated for each antigen (OD calibrator/OD cutoff) and a ratio > 1 (OD sample \times CF/OD calibrator) was finally defined as cutoff for each antigen.

Addressable laser bead assay. Microspheres embedded with laser reactive dyes (Luminex Corporation, Austin, TX) that were coupled with the purified C22 amino acids of Rib-P were part of a commercial kit (QUANTA Plex 8[™]; INOVA Diagnostics Inc., San Diego, CA). The assay was performed according to the manufacturer's instructions. Briefly, each test serum was diluted to 1/1,000 and 50 μl was added to a well of a microtiter plate, mixed with the antigen-coated beads that were preserved in the well and incubated for 30 min. Then 50 μl of phycoerythrin-conjugated goat anti-human IgG (Jackson ImmunoResearch Laboratories, Inc., WestGrove, PA) was added to each well and incubated for an additional 30 min. The reactivity of the antigen-coated beads was determined on a Luminex 100[™] dual laser flow cytometer (Luminex Corp.). The cutoff for a positive test was based on the reactivity of control samples. The control samples were titrated to provide high, medium, low, and negative values.

Commercially available ELISA-systems

The ELISAs of different suppliers for diagnostic laboratory use were selected according to their antigens—native, recombinant, and synthetic.

MBL. The Ribosomal P ELISA kit (MBL, Code No. 7801E 96 wells; Medical & Biological Laboratories Co., Ltd., Nagoya, Japan) is a semiquantitative detection system for anti-ribosomal antibodies in human serum. The test is based on recombinant ribosomal P0 and a cutoff of 11.6 Units is recommended by the manufacturer based on the 99th percentile of 256 healthy blood donor samples.

Euroimmun. “Antibodies against ribosomal P-Proteins (IgG; Product No. EA 1641-9601 G; INOVA, San Diego, CA)” is a quantitative or semiquantitative ELISA system based on purified ribosomal P proteins from a native source. A cutoff value of 20 RE/mL or a ratio of 1 is recommended by the manufacturer.

INOVA. Ribosome P ELISA (Code No. 708600; INOVA, San Diego, CA) is a semiquantitative test based on a synthetic peptide comprising the 22 C-terminal amino acids, which are shared among the ribosomal P proteins. Samples with less than 20 units are considered negative, 20–39 units as weak positive, 40–79 units as moderate positive, and samples with more than 80 units as strong positive. For the calculations in this study a cutoff of 40 units was selected.

IMMUNOBLOTTING WITH CYTOSOL EXTRACT

The cytosol of HeLa cells was separated from nuclei as previously described and fractionated on a 15% SDS PAGE followed by transfer onto nitrocellulose, and the assay was performed as described previously (5). Briefly, nonspecific binding sites were blocked by overnight (O/N) incubation of the membranes in blocking buffer (BB: 2% milk in TBS) at 4°C. The following steps were done at room temperature (RT). After one washing step for 5 min in TBS-0.2% Tween, membranes were incubated with serum samples at a dilution of 1:500 in BB for 2 hr. Unbound antibodies were removed by three washes in TBS-0.2% Tween. Membranes were transferred to a solution of alkaline phosphatase-conjugated goat anti-human IgG (1:10,000 in BB; Dianova, Hamburg, Germany) and bound antibodies were visualized using blue tetrazolium 5-bromo-4-chloro-indol-3-yl phosphate (NBT/BCIP) as substrate. The results were visually interpreted and quantified into four groups (1 = weak positive to 4 = highly positive).

PREPARATION OF THE RECOMBINANT P0, P1, AND P2 PROTEINS

Recombinant P proteins were produced in insect cells (Sf9) using the baculovirus expression system as described previously (5). Briefly, the full-length cDNA of the P proteins P0, P1, and P2 was isolated from a human liver cDNA library and cloned into the baculo vector pVL1393 (Invitrogen, GmbH, Karlsruhe, Germany). Recombinant proteins were overexpressed for 72 hr after inoculation of 2E+6 Sf9 cells/mL at an MOI of 5. After centrifugation of the cell suspension, the cells were lysed with 6 M Gu/HCl and 10 mM DTT at pH 7.5, and the clarified extract was purified by immobilized metal affinity chromatography (IMAC) (chelating sepharose FF, charged with Ni²⁺ ions).

Desorption of the proteins was performed by step elution with imidazole. Identity and purity of the P-proteins were determined using Western blot analysis and Coomassie blue stained SDS-PAGE. Finally, protein concentrations were determined using BCA reagents (Sigma-Aldrich, St. Louis, MO).

RESULTS

STUDY I—TECHNICAL SENSITIVITY

Anti-Rib-P positive sera (n=30) as determined by immunoblotting were tested in the anti-Rib-P tests obtained from different suppliers. Since anti-Rib-P ELISA systems have been shown to be highly specific but only moderately sensitive, the first goal was to analyze the technical sensitivity and not the technical specificity (9). Furthermore, the commercial assays from each supplier were tested for background reactivity as indicated in the instructions for use. Thus, only two controls were included in the analysis.

Using the different tests—29 out of 30 (96.7%; Euroimmun), 29 out of 30 (96.7%; MBL), 28 out of 30 (93.3%; INOVA), 30 out of 30 (100%; QuantaPlex™ Ribo-P), 29 out of 30 (96.7%; Varelisa® C22), and 30 out of 30 (100%; Varelisa® P0, P1, and P2, respectively), samples were found positive for anti-ribosomal antibodies using the manufacturer’s suggested cutoff values (see Table 1). The test from Euroimmun, INOVA, and MBL failed to detect the same sample (no. 22) that had weak reactivity in the other tests and moderate reactivity when tested by immunoblot. Sample 23 was slightly below the cutoff (39.4 units) in the INOVA ELISA, and sample 4 tested negative with the Varelisa® C22 kit but was positive with all the other tests. No false positive results were recorded in the assays used in this study.

The correlation between the Rib-P tests from different suppliers showed statistical R² values ranging from 0.73 (INOVA QuantaPlex™ Ribo-P vs. MBL) to 0.95 (Euroimmun vs. MBL). Surprisingly, the correlation value between the QuantaPlex™ Ribo-P results and the results from the INOVA ELISA was 0.74, although both tests are based on the same antigen (see Fig. 1). The results of the different Varelisa® tests (P0, P1, P2, and C22) were highly correlated and varied between R²=0.87 (P0 vs. C22) and 1.0 (P1 vs. P2) (data not shown).

Although the qualitative evaluation of the results of the ELISA systems and the immunoblot was in a good agreement, significant variations could be observed by quantitative interpretation of the results. Sample 4, for example, showed weak reactivity in immunoblotting but was highly positive in the ELISA from INOVA (185 units). In contrast, sample 23 was only moderately

TABLE 1. Reactivity of anti-Rib-P sera in the different tests

Sera ID	Commercial ELISA systems				Varelisa [®]				IB>0 ^a
	Euroimmun ratio 1 ^a /20 RE/mL	MBL U/mL 11.6 ^a	INOVA units 40 ^a	Luminex INOVA ratio 1 ^a	C22 U/mL 6.1 ^a	P0 U/mL 6.1 ^a	P1 U/mL 6.1 ^a	P2 U/mL 6.1 ^a	
1	9.1	218.8	201.7	14.5	2250.0	1180.0	1690.0	1230.0	3
2	5.6	144.0	163.7	8.1	75.7	73.8	81.4	83.6	3
3	7.9	123.6	200.3	13.5	373.0	429.0	671.0	656.0	3
4	2.2	23.8	185.0	11.3	5.0 ^b	14.9	23.0	36.7	1
5	4.4	75.9	140.3	17.1	61.0	62.8	73.9	71.2	3
6	5.1	62.9	178.1	8.0	49.4	57.7	100.0	124.0	2
7	6.8	111.1	165.2	12.0	309.0	218.0	255.0	197.0	3
8	7.7	169.3	196.6	24.9	379.0	286.0	409.0	441.0	3
9	8.9	246.4	212.4	12.7	754.0	859.0	633.0	778.0	3
10	7.5	186.6	197.7	11.7	272.0	271.0	189.0	204.0	3
11	3.6	69.1	88.6	7.5	28.9	37.7	44.3	47.1	2
12	2.8	71.5	138.6	5.8	20.6	29.5	28.4	30.5	2
13	6.6	125.1	184.0	13.1	198.0	202.0	261.0	243.0	3
14	6.3	130.4	180.6	12.2	185.0	197.0	253.0	241.0	3
15	5.2	115.8	173.2	14.2	21.4	75.0	107.0	124.0	3
16	3.4	86.8	148.1	5.3	19.8	32.9	43.5	40.9	2
17	6.0	136.0	163.7	10.1	85.0	103.0	112.0	99.5	3
18	5.3	118.4	155.1	19.9	88.5	88.5	94.0	116.0	3
19	5.8	100.3	149.8	3.0	36.9	58.0	98.0	99.9	3
20	7.8	169.5	205.6	16.8	294	194.4	235.6	263.2	3
21	7.1	145.5	199.4	15.7	500	204.5	262.5	242.5	3
22	1.0 ^b	10.8 ^b	20.3 ^b	1.5	8	8.4	10.8	11.9	2
23	2.1	18.7	39.4 ^b	3.8	12.6	11.1	15.4	16.4	3
24	7.4	154.6	204.1	9.8	298	669	383	937	3
25	3.9	76.0	54.3	10.0	44.8	111	61	129	3
26	7.6	143.3	192.0	8.7	327	693	522	510	3
27	7.2	148.5	203.0	23.2	167	309	196	507	3
28	9.1	238.7	218.9	27.1	10000	10000	10000	10000	4
29	3.2	38.4	75.1	2.1	39.4	51.2	61.7	58.4	3
30	1.6	55.3	65.8	2.4	16.5	11.4	15.3	20.2	2
31	0.1	0.6	3.0	0.2	1.9	0.2	0.1	0.4	0
32	0.1	1.1	5.6	0.2	5.5	0.4	0.2	0.6	0

^aSuggested cutoff values.^bTested false negative.

reactive in the ELISA systems and the QuantaPlex[™] Ribo-P test but highly positive in immunoblotting (see Table 1).

STUDY II—CLINICAL ACCURACY

Sera from 50 unselected SLE patients and various control sera (n=100) were tested in the anti-Rib-P assays from different suppliers including the ELISA tests from Euroimmun, MBL and INOVA. Furthermore, all sera were also tested in the new QuantaPlex[™] assay (INOVA) and Varelisa[®] Rib-P profile test (Pharmacia Diagnostics, Freiburg, Germany). Using the cutoff values suggested by the suppliers of the commercial ELISA tests—6 out of 50 (12%; Euroimmun), 7 out of 50 (14%; INOVA), and 10 out

of 50 (20%; MBL), patient sera tested positive for Rib-P antibodies (see Table 2). None of the disease controls displayed anti-Rib-P reactivity under the respective conditions in the ELISA assays of Euroimmun and INOVA, resulting in a specificity of 100%. The positive predictive value (PPV) and the negative predictive value (NPV), as well as the test efficiency (TE), were calculated at 100%, 69.4%, and 70.7% for Euroimmun and at 100%, 69.9%, and 71.3% for INOVA. Five control sera tested positive for anti-Rib-P antibodies using the ELISA test from MBL. Thus, the specificity, PPV, NPV, and TE of the MBL Rib-P test was calculated at 95%, 66.7%, 70.4%, and 70.0%, respectively. Using a cutoff of 1 (ratio = value sample/value low positive) as suggested by the manufacturer for the QuantaPlex[™] test, 5 out of 50 patients with SLE

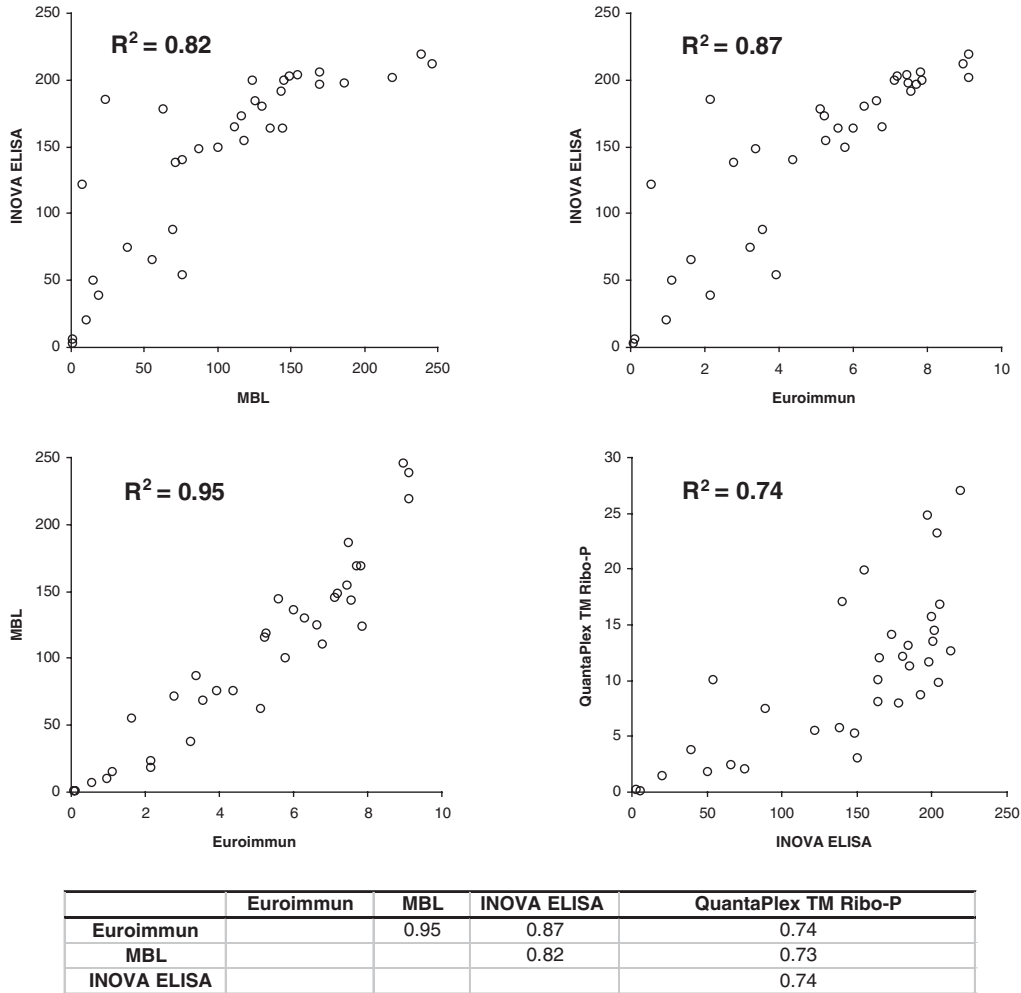


Fig. 1. Correlation of the different Rib-P tests for diagnostic laboratory use. The results of the assays that used the identical 30 Rib-P positive sera and two controls were used to generate correlation diagrams and to calculate the statistical correlation values (R^2) according to Pearson. Those R^2 values varied between 0.73 (QuantaPlex™ Ribo-P vs. MBL) and 0.95 (MBL vs. Euroimmun).

tested positive for anti-ribosomal antibodies. None of the control sera had assay values above the suggested cutoff, resulting in a sensitivity of 10% and a specificity of 100% for lupus. The PPV, NPV, and TE were calculated at 100%, 69.4%, and 70.7%, respectively (see Table 2).

Since some sera were either positive with one of the recombinant Rib-P proteins or with the C22 peptide, we developed a Varelisa® Rib-P profile assay that allows for the semiquantitative determination of antibodies to all of the above-mentioned antigens (C22, P0, P1, and P2). Using the Varelisa® Rib-P profile 8 out of 50 (16%) SLE patients tested positive for the C22 peptide and 9 out of 50 (18%) for the recombinant proteins, respectively. The specificity was 100% for all antigens. PPV, NPV, and TE were 100%, 70.4%, and 72% for C22 and 100%, 70.9%,

and 72.7% for all three recombinant proteins (see Table 2).

Although the number of positive sera was found to be greater when the recombinant proteins (9 out of 50) were used and compared to the C22 peptide (8 out of 50), some sera only reacted with the peptide. Thus, 12 out of 50 (24%) of the SLE sera were positive for at least one of the antigens C22, P0, P1, or P2 in the Varelisa® Rib-P profile resulting in an increased PPV, NPV, and TE of 100%, 72.5%, and 74.7%, respectively (see Table 2).

The results of all Rib-P assays were subjected to a comparative Receiver Operating Characteristic (ROC) analysis, which showed that the discrimination between positive sera and controls as expressed by the area under the curve (AUC) varied from 0.577 (QuantaPlex™ Ribo-P) to 0.846 (INOVA ELISA). Results are summarized in Fig. 2.

TABLE 2. Clinical accuracy of anti-Rib-P antibody tests from different suppliers

	Cutoff	Sensitivity %	Specificity %	PPV %	NPV %	Efficiency %	AUC ^a
Varelisa C22	1.0 (ratio)	16	100	100	70.4	72	0.605
Varelisa P0	1.0 (ratio)	18	100	100	70.9	72.7	0.672
Varelisa P1	1.0 (ratio)	18	100	100	70.9	72.7	0.682
Varelisa P2	1.0 (ratio)	18	100	100	70.9	72.7	0.686
Varelisa Rib-P profile	1.0 (ratio)	24	100	100	72.5	74.7	–
INOVA ELISA	40 units ^b	14	100	100	69.9	71.3	0.846
	18.6 units	18	100	100	70.9	72.7	–
QuantaPlex™ Rib-P	1.0 (ratio)	10	100	100	69.4	70.7	0.577
	0.37 (ratio)	18	100	100	70.9	72.7	–
Euroimmun	1.0 or 20 RE/ml	12	100	100	69.4	70.7	0.696
MBL	11.6 U/ml ^c	20	95	66.7	70.4	70	0.651
	19.6 U/ml	12	100	100	69.4	70.7	–

^aArea under the curve (AUC) calculated using the analyze-it software.
^bModerate positive.
^c99% percentile of 256 healthy blood donor samples.
^dVarelisa Rib-P profile containing C22, P0, P1, and P2 in separate wells.

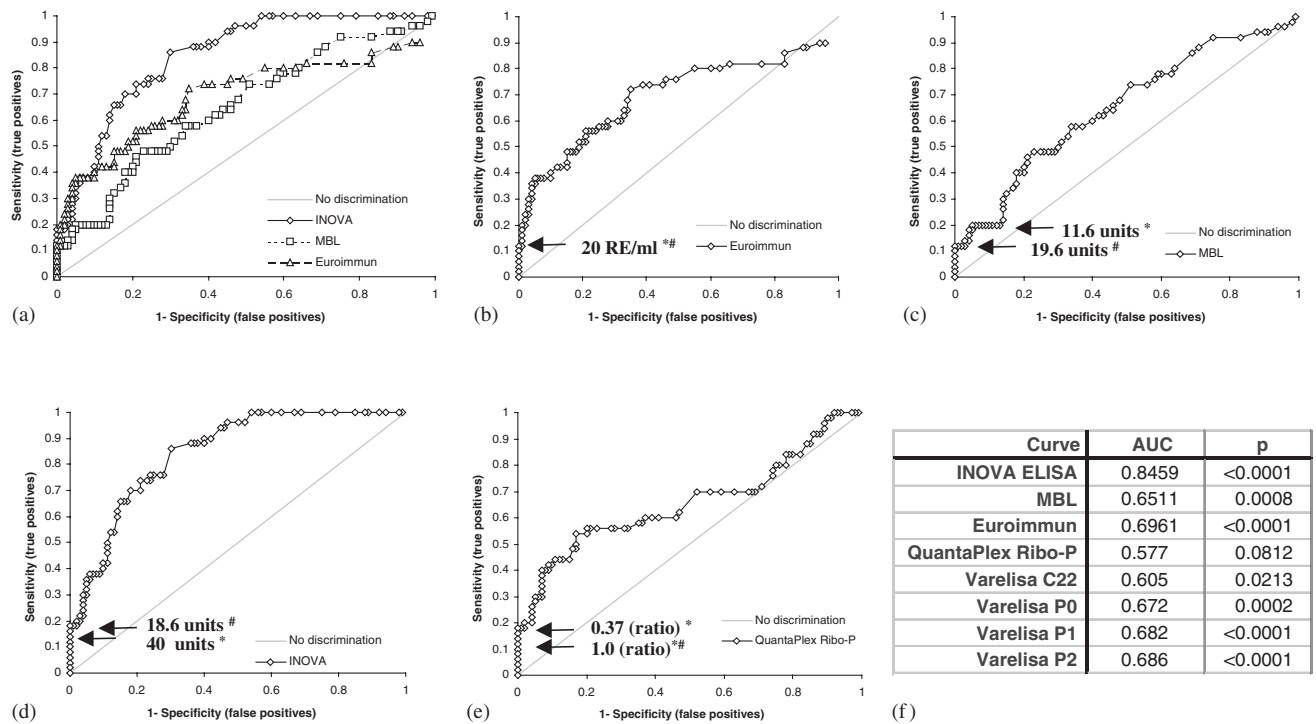


Fig. 2. Comparative receiver operator characteristics (ROC) analysis of the anti-Rib-P tests from different suppliers (a–f). The results of the clinical part of this study were used to calculate ROC curves. Within the accurate area, the curves of all tests are similar. The cut-off values recommended by the manufacturers (*) and the adjusted values (#) are indicated by the arrows. Using the analyze-it software the area under the curve (AUC) and the statistical *P* values of all assay tests were calculated (f).

This data was subsequently used to optimize the cutoff value of each kit for the patient group investigated in this study. Using the adjusted cutoff values for the patient group tested in this study, the assay performance of the INOVA ELISA, MBL ELISA, and INOVA QuantaPlex™ Ribo-P test was increased.

The most significant improvement could be achieved for the QuantaPlex™ Ribo-P test. At a cutoff of 0.37 (ratio) the sensitivity of this assay increased to 18% without a loss in specificity, and thus the PPV, NPV, and TE were found at 100%, 70.9%, and 72.7%, respectively (see Table 2). No improvement could be achieved for the test from Euroimmun.

DISCUSSION

DETECTION OF ANTI-RIBOSOMAL-P PROTEIN ANTIBODIES

This comparative technical study, using kits from several suppliers, has shown that all assays used yielded a high technical sensitivity ranging from 93.3–100%. Furthermore, as revealed by the correlation data, it became evident that the detection of anti-ribosomal antibodies strongly depends on the detection system and on the nature of the antigen. Although both the QuantaPlex™ Ribo-P addressable laser bead and the INOVA ELISA are based on the same antigen (C22), a relatively poor correlation value of $R^2 = 0.74$ was observed in this study. This indicates that not only does the specificity of anti-ribosomal antibodies play an important role in the test results, but the binding properties such as the affinity of those antibodies does also.

Although the QuantaPlex™ Ribo-P test displayed the lowest AUC value, the discrimination between SLE patients and controls within the critical area of the test was satisfactory. Looking at the ROC curves we found that the suggested cutoff values of the MBL ELISA, the INOVA ELISA, and the QuantaPlex™ Ribo-P test were suboptimal for the patient group used in this study. Adjusted cutoff values increased the sensitivities, and thus the test efficiency, of both INOVA Rib-P assays. In contrast, we recommend a higher cutoff value (19.6 units) for the test from MBL which results in a 100% specificity of the assay. Based on these findings we propose that the cutoff values should be verified in an extended multicenter study.

Although the MBL Rib-P test and the Varelisa® P0 assay are both based on recombinant P0, the sensitivity of the Varelisa® is significantly higher (18% vs. 12% for the MBL test). This obvious lack of sensitivity of the MBL kit may be related to the technical production protocol of the test or impurity of the recombinant antigen. The results of the comparative study using anti-Rib-P assays of different suppliers showing sensitivities between 10% and 24% are in a good agreement to previous studies with research assays (5,9,12–14,17).

Various techniques, in combination with a variety of different antigens, have been proposed for the detection of anti-ribosomal antibodies: immunoblotting with

native antigens from different sources, purified or recombinant proteins, and synthetic peptides (3,9, 11–14,17). A recent study has confirmed the high efficiency of the immunoblot technique compared to peptide ELISAs based on the C22 peptide and compared to a MAP construct (9). An obvious advantage of the immunoblot with native antigen is that the detection of antibodies to the individual Rib-P proteins P0, P1, and P2 is possible. This feature has been incorporated into the Varelisa® Rib-P profile, which contains the C22 peptide in addition to the recombinant antigens. Taking this approach has increased the sensitivity from 16% (C22) or 18% (recombinant) to 24% for SLE (≥ 1 positive). Thus, the best method to detect anti-ribosomal antibodies appears to be the combination of different antigens. Whether the test performance can further be increased by adding the MAP construct and native antigens to the profile has to be investigated.

Advances in multiplex technologies and microarrays allow for the development of sophisticated profile assays containing a number of different antigens (26–28). This may improve the diagnosis of a variety of disorders, especially of autoimmune diseases, since no highly sensitive marker is available for most of those disorders. For example, the diagnosis of SLE might be improved by providing an antigen array that includes different ribosomal antigens in combination with dsDNA and Sm antigens.

International standardization of laboratory testing of Rib-P antibodies has yet to be fully realized, and a reference anti-Rib-P sera for calibration of “in house” control sera and quantification of antibody levels are still not available (29). Based on the results of the first part of this study showing the concordant results with the tests from different suppliers, we suggest that the development of an international standardization is mandatory. The inclusion of an anti-Rib-P serum into the reference panel used for the determination of antinuclear antibodies (ANA) and ANA subsets, which are available from the Centers for Disease Control and Prevention (CDC) in Atlanta, would represent an important step towards standardization (30).

The development and production of a diagnostic kit according to the “good manufacturing practice (GMP)” guidelines is not a trivial task. After the proof of principle of the respective research test, several laborious and time consuming evaluation and validation steps have to be performed. The test has to be scaled up from a laboratory or research environment to the respective production scale that depends on sales forecasts as well as on the stability of the kit. Therefore, promising research kits often lose accuracy during the transfer process from the development phase to launch

of the commercial product (Mahler, unpublished data). The anti-Rib-P test evaluated in this study shows a comparable performance as the research kits from previous studies (1,5,9,11–14).

SUMMARY

In summary, we have found that the detection of anti-Rib-P antibodies strongly depends both on the nature and quality of the antigen and on the detection system. Although the assays from all suppliers used in this study performed well in the technical part of this study, relatively poor correlation and significant differences in the clinical accuracy were found.

CONCLUSION

Based on these findings we conclude that anti-Rib-P assays should be standardized at an international level. Furthermore, we conclude that the Varelisa[®] Rib-P profile and the addressable laser bead Rib-P assays represent promising tools and platforms for the detection of anti-Rib-P antibodies in the future.

ACKNOWLEDGEMENTS

The authors acknowledge the technical assistance of Joan Miller and Laura Stinton at the University of Calgary.

REFERENCES

- Bonfa E, Golombek SJ, Kaufman LD, et al. Association between lupus psychosis and anti-ribosomal P protein antibodies. *N Engl J Med* 1987;317:265–271.
- Elkon K. Ribosomal RNP. In: Van Venrooij WJ, Maini RN, editors. *Manual of biological markers of disease*. Dordrecht: Kluwer Academic Publishers; 1994. B2.5:1–11.
- Greenwood DL, Gitlits VM, Alderuccio F, Sentry JW, Toh BH. Autoantibodies in neuropsychiatric lupus. *Autoimmunity* 2002;35:79–86.
- Hasler P, Brot N, Weissbach H, Parnassa AP, Elkon KB. Ribosomal proteins P0, P1, and P2 are phosphorylated by casein kinase II at their conserved carboxyl termini. *J Biol Chem* 1991;266:13815–13820.
- Mahler M, Kessenbrock K, Raats J, Williams R, Fritzler MJ, Bluthner M. Characterization of the human autoimmune response to the major C-terminal epitope of the ribosomal P proteins. *J Mol Med* 2003;81:194–204.
- Hasler P, Brot N, Weissbach H, et al. The effect of phosphorylation and site-specific mutations in the immunodominant epitope of the human ribosomal P proteins. *Clin Immunol Immunopathol* 1994;72:273–279.
- Teh LS, Lee MK, Wang F, et al. Antiribosomal P protein antibodies in different populations of patients with systemic lupus erythematosus. *Br J Rheumatol* 1993;32:663–665.
- Arnett FC, Reveille JD, Moutsopoulos HM, Georgescu L, Elkon KB. Ribosomal P autoantibodies in systemic lupus erythematosus. Frequencies in different ethnic groups and clinical and immunogenetic associations. *Arthritis Rheum* 1996;39:1833–1839.
- Isenberg DA, Garton M, Reichlin MW, Reichlin M. Long-term follow-up of autoantibody profiles in black female lupus patients and clinical comparison with Caucasian and Asian patients. *Br J Rheumatol* 1997;136:229–233.
- Ghirardello A, Caponi L, Franceschini F, et al. Diagnostic tests for antiribosomal P protein antibodies: a comparative evaluation of immunoblotting and ELISA assays. *J Autoimmun* 2002;19:71–77.
- Bonfa E, Gaburo Junior N, Tavares AV, Cossermelli W. Comparison of five methods for the detection of antiribosomal P protein antibody. *Braz J Med Biol Res* 1994;27:637–643.
- Caponi L, Pegoraro S, Di Bartolo V, Rovero P, Revoltella R, Bombardieri S. Autoantibodies directed against ribosomal P proteins: use of a multiple antigen peptide as the coating agent in ELISA. *J Immunol Methods* 1995;179:193–202.
- Yoshio T, Masuyama J, Ikeda M, et al. Quantification of antiribosomal P0 protein antibodies by ELISA with recombinant P0 fusion protein and their association with central nervous system disease in systemic lupus erythematosus. *J Rheumatol* 1995;22:1681–1687.
- Rayno K, Reichlin M. Evaluation of assays for the detection of autoantibodies to the ribosomal P proteins. *Clin Immunol* 2000;95:99–103.
- Sato T, Uchiumi T, Ozawa T, et al. Autoantibodies against ribosomal proteins found with high frequency in patients with systemic lupus erythematosus with active disease. *J Rheumatol* 1991;18:1681–1684.
- Martin AL, Reichlin M. Fluctuations of antibody to ribosomal P proteins correlate with appearance and remission of nephritis in SLE. *Lupus* 1996;5:22–29.
- Reichlin M, Wolfson-Reichlin M. Evidence for the participation of anti-ribosomal P antibodies in lupus nephritis. *Arthritis Rheum* 1999;42:2728–2729.
- Torre JC, Mozo L, Suarez A, Ramos E, Gutierrez C. Antibodies to ribosomal P proteins and hepatic damage in undifferentiated CTD. *Ann Rheum Dis* 1996;55:562–563.
- Schneebaum AB, Singleton JD, West SG, et al. Association of psychiatric manifestations with antibodies to ribosomal P proteins in systemic lupus erythematosus. *Am J Med* 1991;90:54–62.
- Reichlin M, Broyles TF, Hubscher O, et al. Prevalence of autoantibodies to ribosomal P proteins in juvenile-onset systemic lupus erythematosus compared with the adult disease. *Arthritis Rheum* 1999;42:69–75.
- Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982;25:1271–1277.
- Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315–324.
- Tanimoto K, Nakano K, Kano S, et al. Classification criteria for polymyositis and dermatomyositis. *J Rheumatol* 1995;22:668–674.
- Sharp GC, Irvin WS, Tan EM, Gould RG, Holman HR. Mixed connective tissue disease—an apparently distinct rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen (ENA). *Am J Med* 1972;52:148–159.
- Vitali C, Bombardieri S, Moutsopoulos HM, et al. Preliminary criteria for the classification of Sjogren's syndrome. Results of a prospective concerted action supported by the European community. *Arthritis Rheum* 1993;36:340–347.
- Robinson WH, DiGennaro C, Hueber W, et al. Autoantigen microarrays for multiplex characterization of autoantibody responses. *Nat Med* 2002;8:295–301.

27. Robinson WH, Steinman L, Utz PJ. Proteomics technologies for the study of autoimmune disease. *Arthritis Rheum* 2002;46:885–893.
28. Fritzler MJ. New technologies in the detection of autoantibodies. In: Conrad K, Fritzler MJ, Meurer M, Sack U, Shoenfeld Y, editors. *Autoantigens, autoantibodies, autoimmunity*. Lengerich: Pabst Scientific Publishers; p 50–63.
29. Fritzler MJ, Wiik A, Fritzler ML, Barr SG. The use and abuse of autoantibody kits. *Arthritis Res Ther* 2003;5:192–201.
30. Smolen JS, Butcher B, Fritzler MJ, et al. Reference sera for antinuclear antibodies. II. Further definition of antibody specificities in international antinuclear antibody reference sera by immunofluorescence and western blotting. *Arthritis Rheum* 1997;40:413–418.
31. Elkon K, Bonfa E, Llovet R, Danho W, Weissbach H, Brot N. Properties of the ribosomal P2 protein autoantigen are similar to those of foreign protein antigens. *Proc Natl Acad Sci U S A* 1988;85:5186–5189.
32. Nojima Y, Minota S, Yamada A, Takaku F, Aotsuka S, Yokohari R. Correlation of antibodies to ribosomal P protein with psychosis in patients with systemic lupus erythematosus. *Ann Rheum Dis* 1992;51:1053–1055.