FACULTY OF MEDICINE | UNIVERSITY OF CALGARY

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Limited reliability of the indirect immunofluorescence technique for the detection of anti-Rib-P antibodies

Fritzler MJ[1], Ngo J[1], Hou H[1], Mahler M[2]

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

Background: Autoantibodies (aab) to the three ribosomal P proteins, P0, P1 and P2 (referred to as Rib-P) are considered a relatively specific serological marker for systemic lupus erythematosus (SLE). Although more than 25 years has lapsed since their first description, 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. The objective of this study was to analyse the sensitivity of IIF for the detection of anti-Rib-P antibodies.

Methods: Sera that were positive for anti-Rib-P as detected by an addressable laser bead immunoassay (ALBIA; QuantaPlex ENA8, INOVA, US) collected between 2003 and 2007 at the Mitogen Advanced Diagnostics Laboratory (Calgary, Canada) were retrospectively analysed for aab by IIF (HEp-2000, ImmunoConcepts, USA) and read at serum dilutions of 1/160 and 1/640 by two experienced technologists (> 5 years experience) who had no knowledge of the ALBIA results. Randomly selected samples (n=51) with anti-Rib-P reactivity (ALBIA) were tested by Ribosomal P ELISA (Dr. Fooke Laboratorien GmbH).

Results and findings: Over the audit period, anti-Rib-P reactivity was identified by ALBIA in 345/~20,000 (~2%) serum samples. Only 35/345 (10.1%) of anti-Rib-P positive sera displayed an IIF cytoplasmic staining pattern (CP) compatible with the presence of anti-Rib-P aab. When only moderate and high titre anti-Rib-P positive samples were considered, the percentage of samples that had the typical IIF CP increased. 26/145 (17.9%) of moderate and high titre anti-Rib-P and 18/82 (22.0%) of high titre samples showed CP. 8/345 (2.3%) anti-Rib-P positive samples were tested negative (no staining pattern) in IIF. Six of those samples had low and two had high titres of anti-Rib-P aab by ALBIA. 45/345 (13%) were “monospecific” for anti-Rib-P aab when the ALBIA results were considered. In a randomly selected cohort of ALBIA anti-Rib-P positive samples anti-Rib-P reactivity was confirmed in 71% by ELISA (cut-off 1 RU).

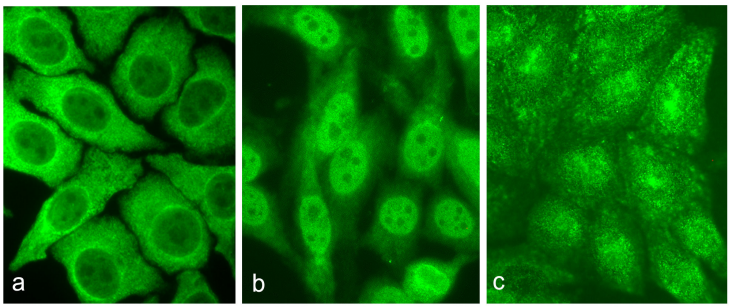
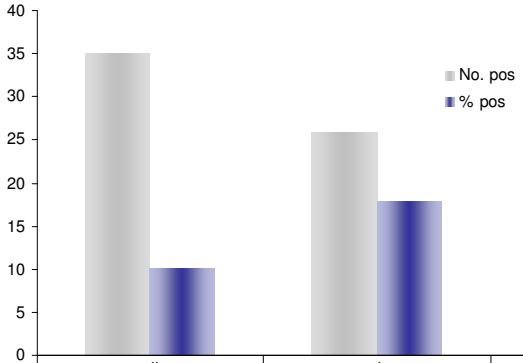


Figure 1 Indirect immunofuorescence (IIF) staining pattern of anti-Rib-P positive samples. An anti-Rib-P positive serum that did not have autoantibodies to other known antigens was tested at a 1:500 dilution on slides from three different suppliers.



	all	> moderate	> high
No. pos	35	26	18
% pos	10.1	17.9	22

Figure 2 Number and percentage of anti-Rib-P positive samples with cytoplasmic IIF staining pattern (CP) on HEp-2 cells. Number and percentage of anti-Rib-P positive samples with CP in all ALBIA anti-Rib-P positive samples (n=345), moderate and high positives (n=145) and high positives only (n=82).

Conclusion: Based on these findings, we conclude that routine screening for Rib-P aab by IIF on HEp-2 cell substrates has low sensitivity and, thus, limited reliability for the detection of anti-Rib-P in a routine clinical laboratory setting. Between 78% and 90% of samples with anti-Rib-P reactivity depending on the anti-Rib-P titre determined by ALBIA were not accompanied by a CP on commercially prepared HEp-2 cells.

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