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Evaluation of a novel sensitive and specific Lateral Flow Assay (LFA) for the detection of anti-dsDNA antibodies

Anti-dsDNA LFA

N. Offermann, M. Petschinka and M. Fooke
Dr. Fooke Laboratorien GmbH, Neuss, Germany

Background: The presence of anti-double-stranded DNA (anti-dsDNA) IgG antibodies is considered highly specific for the diagnosis of systemic lupus erythematosus (SLE). Anti-dsDNA antibodies can be detected in 20 - 80% of SLE patients. Due to the fact that the titer of anti-dsDNA antibodies correlates with disease activity the periodic determination of these antibodies is a must for monitoring SLE patients [1-3] .
The current diagnostics for antibodies to dsDNA rely on a combination of IFA and ELISA. A new, innovative platform is the anti-dsDNA LFA, a highly sensitive and specific rapid test for the detection of IgG antibodies to dsDNA. Together with a new handheld scanning device this technology provides sensitive, specific, fast and economic diagnosis of anti-dsDNA antibodies without the need for extensive laboratory equipment.

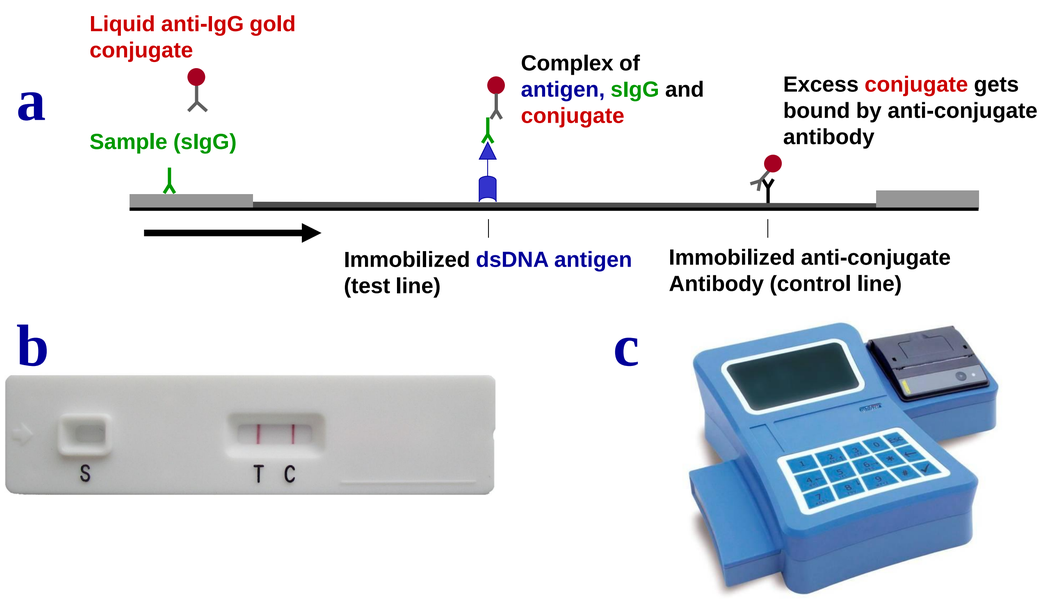

Figure 1 Principle of anti-dsDNA LFA (a), LFA cassette (b) and opTrilyzer plus (c)

Methods: Serum samples from SLE patients (n=69), control sera from healthy donors (n=10) and autoimmune disease controls (n=5) were tested by commercial anti-dsDNA ELISA kits (Dr. Fooke Labs, Orgentec and a high avidity ELISA from Inova). Results of the anti-dsDNA LFA were measured using a handheld scanning device and compared to ELISA results. Statistics were done with Analyze-it for Microsoft-Excel.

Results and findings: Comparison between the novel anti-dsDNA LFA and anti-dsDNA ELISA from Dr. Fooke Labs revealed an excellent Area Under the Curve (AUC) value of 0.98 in the ROC (Receiver Operating Curve) analysis for all 84 serum samples. This corresponds to a technical sensitivity and specificity of 98.5% / 94.7%, the cut-off was set at 100 RU. Using the same cut-off the ROC Analysis between LFA and Orgentec ELISA shows an AUC of 0.88 and a sensitivity / specificity of 100% / 70.4% (see figure 2).

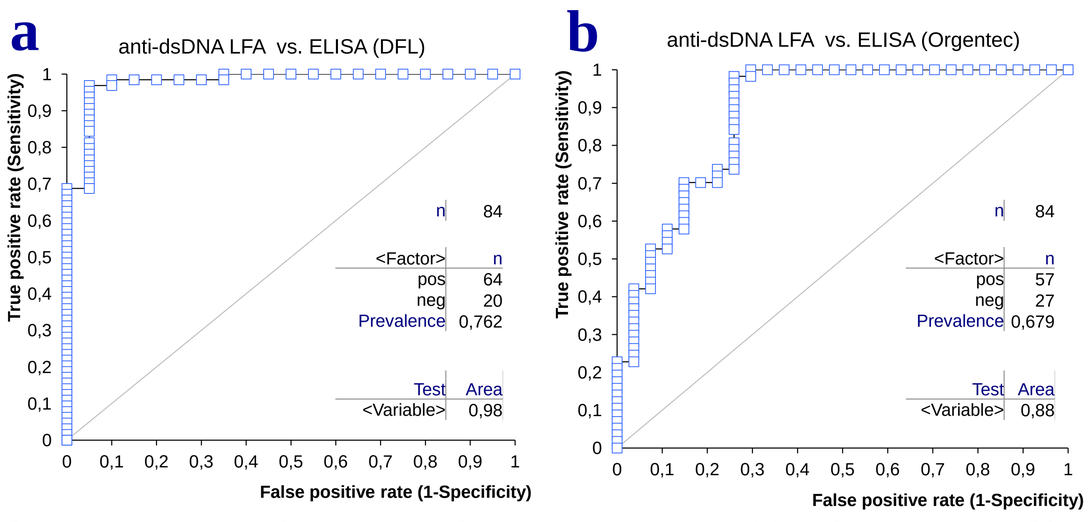

Figure 2 ROC Analysis of anti-dsDNA LFA vs. DFL (a) and vs.Orgentec (b)

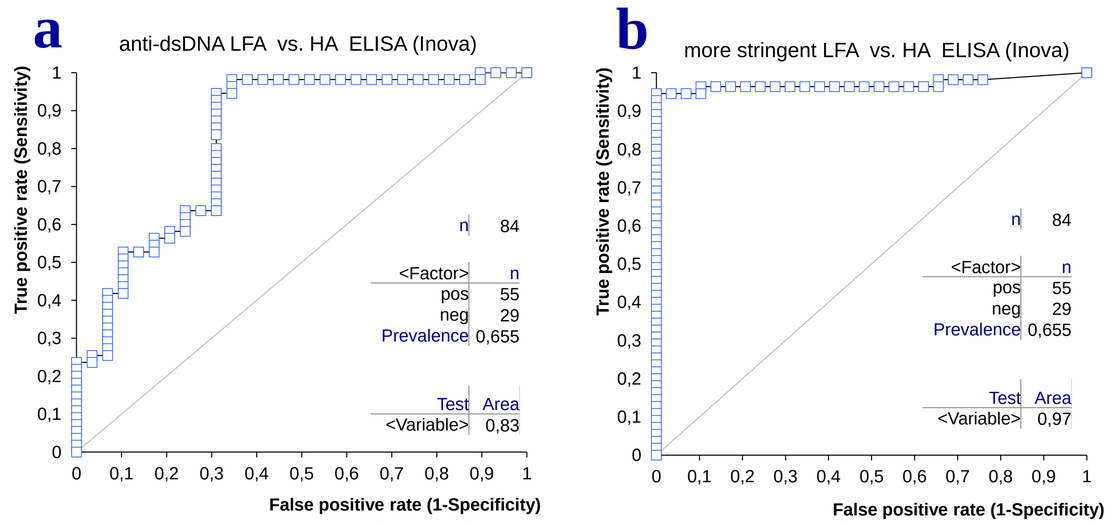

Figure 3 ROC Analysis of anti-dsDNA LFA vs. HA ELISA from Inova (a) and anti-dsDNA LFA with more stringent assay conditions vs. HA ELISA from Inova (b)

ROC Analysis between anti-dsDNA LFA and a High Avidity (HA) ELISA from Inova shows only a moderate AUC of 0.83 (see figure 3a). Therefore we developed a second set up with more stringent assay conditions. With this set up we intend to detect only the “high avidity” IgG antibodies, which are associated with the disease activity of SLE patients. Comparison between the more stringent anti-dsDNA LFA and HA ELISA shows an excellent AUC of 0.97 and a sensitivity and specificity of 92.7% and 100% (see figure 3b).
Overall agreements between the two LFA set ups and four different ELISAs range from 81.0% to 97.6% (see table 1). The “standard” anti-dsDNA LFA shows the best agreement with two ELISAs (grey highlighted), which detect all anti-dsDNA IgG antibodies. The agreement of the more stringent anti-dsDNA LFA is significantly higher with the high avidity ELISAs (blue highlighted) than with the standard ELISAs.
Control sera with IgG to single-stranded DNA (ssDNA) were tested negative in both anti-dsDNA LFA set ups (Data not shown).

				Overall Agreement (%)	
Manufacturer	**High Avidity**	**negative (n=)**	**positive (n=)**	**anti-dsDNA LFA**	**more stringent anti-dsDNA LFA**
Dr. Fooke Labs	No	20	64	97.6	81.0
Dr. Fooke Labs	Yes	33	51	83.3	88.1
Orgentec	No	27	57	90.5	83.3
Inova	Yes	29	55	85.7	95.2

Table 1 Overall agreement between two different set ups of the anti-dsDNA LFA (cut-off 100 RU) and 4 ELISAs for detecting anti-dsDNA antibodies

Conclusion: The anti-dsDNA LFA shows a good diagnostic performance in comparison to commercial ELISA tests (Dr. Fooke Labs and Orgentec), therefore the anti-dsDNA LFA is an excellent rapid test for monitoring SLE patients. In contrast, the LFA with more stringent assay conditions seems to detect predominantly the high avidity IgG antibodies to dsDNA.
Both set ups provide a sensitive and specific diagnosis within a short incubation time of only 20-30 min and without the need for extensive laboratory equipment.

References:
1. Brenda B Suh-Lailam, Tyson R Chiaro, Wayne Davis K, Andrew R Wilson, Anne E Tebo: **Evaluation of a high avidity anti-dsDNA IgG enzyme-linked immunosorbent assay for the diagnosis of systemic lupus erythematosus**. *Int J Clin Exp Pathol* 2011;4(8): 748-754
2. David Launay, Jean Schmidt, Sébastien Lepers, Tristan Mirault, Marc Lambert , Xavier Kyndt, Dominique Reumaux, Alain Duhamel, Eric Hachulla, Pierre-Yves Hatron, Lionel Prin, Sylvain Dubucquoi: **Comparison of the Farr radioimmunoassay, 3 commercial enzyme immunoassays and Crithidia luciliae immunofluorescence test for diagnosis and activity assessment of systemic lupus erythematosus.** *Clinica Chimica Acta* 2010; 411: 959-964
3. Tyson R. Chiaro, Kenneth W. Davis, Andrew Wilson, Brenda Suh-Lailam, Anne E. Tebo: **Significant differences in the analytic concordance between anti-dsDNA IgG antibody assays for the diagnosis of systemic lupus erythematosus—Implications for inter-laboratory testing.** *Clinica Chimica Acta* 2011; 412: 1076-1080

11th Dresden symposium on Autoantibodies, September 1-4th 2013, Dresden, Germany

Dr. Fooke Laboratorien GmbH – Habichtweg 16 – 41468 Neuss – Germany
Phone: +49-2131-29840 – Fax: +49-2131-2984-184 – Internet: www.fooke-labs.de – email: information@fooke-labs.de