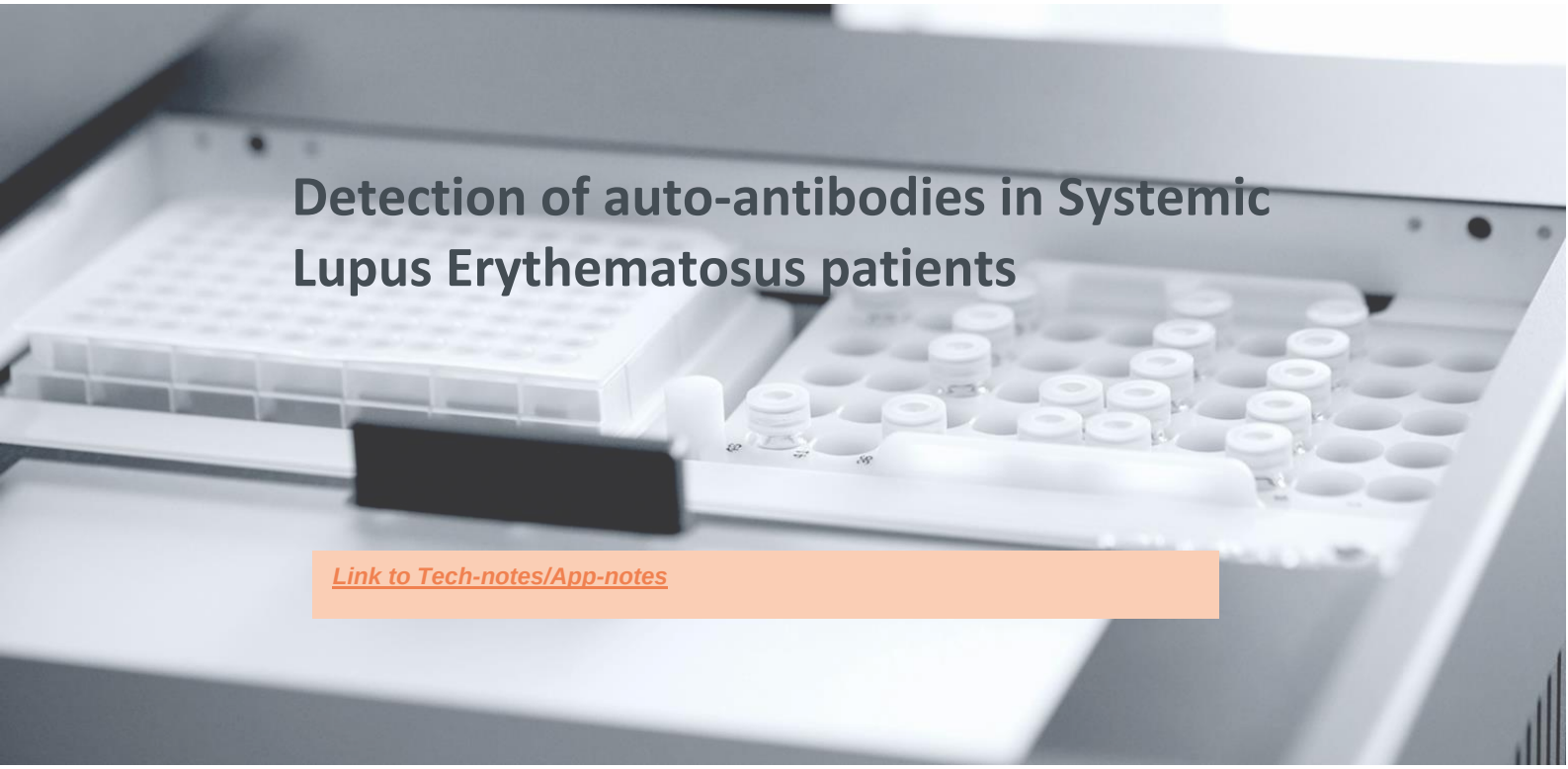

Detection of auto-antibodies in Systemic Lupus Erythematosus patients

Link to Tech-notes/App-notes

VERSION 01 *Morten E. Pedersen*

Introduction

Systemic Lupus Erythematosus (SLE) is an autoimmune disease, where the detection of circulating auto-antibodies against ds-DNA is used as an important diagnosis criterion.
However, current methods for detecting anti-ds-DNA antibodies in blood samples are primarily based on ELISA procedures utilizing surface chemistry (i.e. immobilization of antigen), multiple steps and cumbersome optimization.
Here we present a simple and immobilization-free procedure, termed Flow Induced Dispersion Analysis (FIDA), for detecting auto-antibodies against ds-DNA directly in 85% plasma samples.
FIDA is a new capillary-based technology for measuring binding under native conditions in complex solutions (e.g. plasma). It is based on Taylor dispersion of a ligand (termed indicator, here ds-DNA) which interacts with the analyte of interest (here anti-ds-DNA antibody), in a pressure-driven flow. The indicator appears small (i.e. it has a small hydrodynamic radius) when it is not bound to the antibody. Upon binding, an increase in size is observed, since the complex has a larger hydrodynamic radius. The change in apparent size forms the basis for the accurate measurement of analyte concentration and interaction.

Material & Methods

488 nm Laser-Induced Fluorescence detection. FIDA-coated capillary (i.d.: 75 µm, LT: 75 cm, Leff: 65 cm). 100 mM phosphate buffer pH 7.9 was used as working buffer, three different ds-DNA sequences (32 bp) labelled with atto488 was used as indicators (50-100 nM), a model monoclonal antibody against ds-DNA (0-1000 nM) was spiked into 0-85 % human plasma of a healthy donor to mimic an immune response in patients (healthy control) and used as analyte, 85 % plasma from six SLE patients was used as analyte [1].
Sample analysis was performed by filling the capillary with a zone of analyte, followed by injection of 26 nL of indicator, which was then mobilized with analyte towards the detector at 50 mbar.

Results

The apparent size (hydrodynamic radius) of the ds-DNA indicator was plotted as a function of increasing antibody concentration (0-1000 nM) in 0, 20 and 85% v/v of healthy donor plasma (Figure 1). The obtained dissociation constants (Kd) were 236 nM, 278 nM and 362 nM in 0, 20 and 85% v/v plasma respectively, and thus correlated well.

Fida Biosystems ApS | Fruebjergvej 3 | 2100 Copenhagen | Tel.: +45 53 72 78 70 | www.fidabio.com

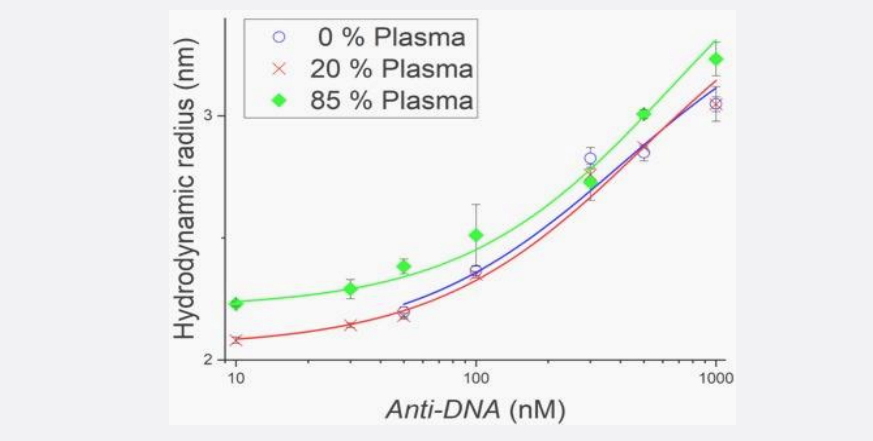

Figure 1: Binding curves of the interaction between ds-DNA-atto488 and monoclonal anti-ds-DNA antibody in 0, 20 and 85 % healthy donor plasma (adapted from [1]).

Plasma samples from six SLE patients were analysed by FIDA using three different DNA sequences (indicators), see Figure 2. The samples were also analysed by ELISA and CLIFT.

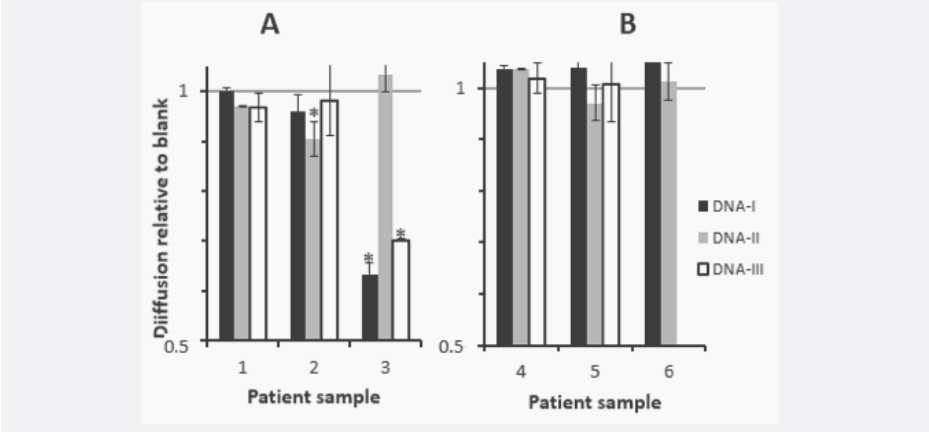

Figure 2. A-samples (positive) and B-samples (negative) when tested with ELISA and CLIFT. FIDA positive samples were patient 2 and 3.

The antibody heterogeneity observed may be used for patient stratification.

References

1. Poulsen NN, Pedersen ME, Østergaard J, et al (2016) Flow-Induced Dispersion Analysis for Probing Anti-dsDNA Antibody Binding Heterogeneity in Systemic Lupus Erythematosus Patients: Toward a New Approach for Diagnosis and Patient Stratification. Anal Chem 88:9056–9061

Fida Biosystems ApS | Fruebjergvej 3 | 2100 Copenhagen | Tel.: +45 53 72 78 70 | www.fidabio.com