Whitepaper

Rapid Determination of IgG Titer and Avidity Using Optical Diffraction

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Background

Serological testing for pathogen-specific antibodies is frequently used in the diagnosis and management of infectious diseases. Generally, the humoral immune system responds to primary infection by producing IgM and IgG antibodies. IgM seroconversion occurs within the first few days of infection onset and levels are detectable for several weeks before disappearing. IgG seroconversion occurs approximately one week following IgM seroconversion but detectable levels can persist for months or years post infection. As such, the presence of IgM is associated with a recent or acute infection while the presence of IgG in the absence of IgM indicates a past or chronic infection. Some infections, however, do not follow this paradigm and detectable IgM levels can persist for several months making serology results difficult to interpret[1].

The polyclonal population of antibodies during the early phases of an infection is comprised of a mixture of high and low affinity IgGs. With somatic hypermutation and repeated antigen exposure, B lymphocytes producing high affinity IgGs are selected for through the process of affinity maturation[2]. This results in an increase in overall IgG avidity during the course of an infection. Therefore, the determination of IgG avidity can assist in the discrimination of patients with recent versus past/distant infections in diseases where IgM levels persist.

The ability to distinguish between recent and past/distant infections has significant clinical utility. In child-bearing women, some infections can lead to undesired sequelae for the fetus if acquired during pregnancy but have no effect on fetal development if acquired prior to pregnancy. For example, Toxoplasma gondii infection can cause major congenital defects or fetal mortality if primary infection occurs during pregnancy and is left untreated[3]. However, if infection is acquired prior to conception, the fetus is not at risk of these deleterious effects. IgM levels in patients with toxoplasmosis can persist for over a year post-infection making it difficult to estimate time of infection onset. Therefore, in these patients, antibody avidity determination can aid in diagnosis and the assessment of risk to the fetus.

Currently, avidity tests are performed using enzyme-linked immunosorbent assays (ELISA) performed on multiple dilutions of the test serum in the presence and absence of a chaotrope to calculate an avidity index. This method is complex, labor intensive and time consuming, making it inappropriate for near patient use.

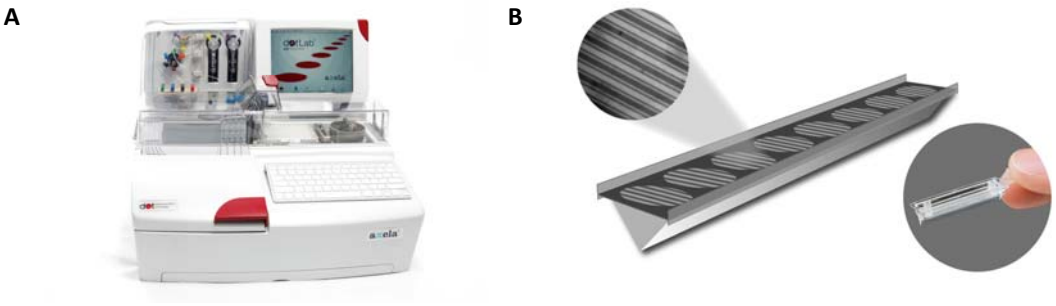
The dotLab® mX System

The dotLab® mX System utilizes diffraction-based optical sensing for the real time, label-free measurement of molecular interactions. The system uses inexpensive, disposable biosensors with coupling reagents (eg: avidin, amine reactive substrates or unique oligonucleotide-based addressing reagents to allow multiplexing) pre-patterned on the surface of 10 µL flow channels forming a diffraction grating. The dotLab® mX instrument illuminates the grating with a laser generating a diffraction image which is monitored by a photodiode detector. Diffractional efficiency increases as molecules bind to the surface resulting in an increase in image intensity. Conversely, molecular dissociation from the surface results in a decrease in image intensity. Therefore, the real time monitoring of molecular interactions through

changes in diffractional efficiency provides information on the quantity and rate of binding and dissociation events. The dotLab® mX System simplifies and automates this analysis using a fully integrated, easy to use, bench top instrument.

Figure 1

(A) The dotLab® mX Instrument: a fully automated, bench-top instrument for real time molecular interaction analysis. (B) Schematic of a dotLab sensor with a contiguous array of capture surfaces (spots) with coupling reagent pre-patterned on the surface forming diffraction gratings.



Here, we demonstrate the use of the dotLab® mX System to rapidly measure antibody titer and avidity in a single assay. The ease and speed of this method make it particularly well suited for researchers looking to understand the value of antibody avidity in developing more accurate and predictive clinical tests.

Materials and Methods

Materials and Reagents

All experiments were performed on the dotLab® mX System (Axela, Inc.) with a running buffer of phosphate-buffered saline containing 0.01% Tween 20 (PBST). Low Cross Buffer (Candor Bioscience GmbH) was used as blocking buffer and sample diluent.

dotLab Analysis

Biotinylated recombinant antigen (4 µg/mL stock) was immobilized on avidin-coated dotLab sensors for 4 minutes with gentle agitation. Antibodies were captured from serum samples (diluted 4-fold in blocking buffer) yielding a binding curve proportionate to the antibody titer. Subsequent incubation with a chaotrope (1M NH_4SCN) was used to dissociate low affinity antibodies form the immobilized antigen. All reagent incubations and wash steps were automated following pre-programmed methods allowing for fully unattended operation.

Data Analysis

The ratio of antibody binding to biotinylated recombinant antigen binding was used as a relative measurement of serum antibody titer. Non-linear fitting of antibody dissociation curves was performed using publically available software packages to generate an observed dissociation rate constant, which was normalized to the antibody titer to yield an avidity index.

Results

The general concepts and conditions of an avidity ELISA test were used to develop the dotLab avidity assay. A major advantage of developing assays on the dotLab® mX System is the ability to monitor the effects of each reagent incubation directly in real time. This allows for the rapid optimization of reagent concentration and incubation times[4].

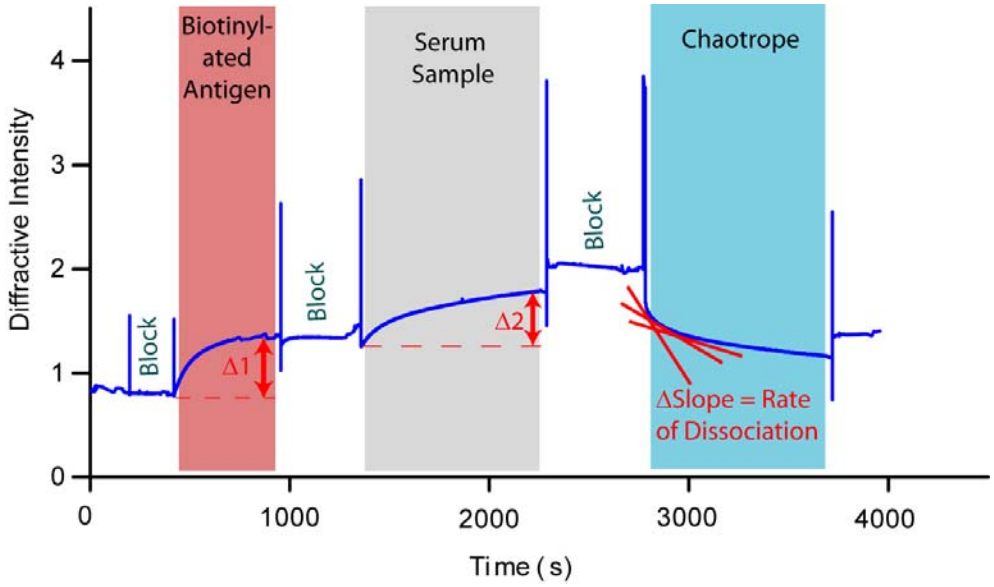
Figure 2 shows a typical result from an avidity assay from which antibody titer and avidity from a sample can be determined in less than one hour. Antibody titer was determined by the ratio of antibody binding signal (Δ2) to the biotinylated antigen binding signal (Δ1). This ratio represents a relative

measurement of antibody titer. Absolute titer, if required, can be obtained from standard curves generated using calibration samples with known antibody concentrations[4].

The observed dissociation rate constant deduced from the chaotrope-induced dissociation curve was used as a measurement of antibody avidity. To account for concentration-dependent effects on dissociation rate, the rate constant was normalized to antibody titer to yield an avidity index. For patient stratification, the avidity indexes of individual samples were compared to a previously determined avidity threshold based on the analysis of well characterized patient samples.

Figure 2

Typical trace from a dotLab avidity assay. The binding of the biotinylated antigen and serum antibodies as well as the chaotrope-induced antibody dissociation can be analysed rapidly in real time.



Summary and Conclusions

- Label-free real time interaction analysis and the ability to sequentially add reagents to sensors on the dotLab® mX System allowed for the measurement of antibody titer and avidity in a single assay that took less than one hour to perform.
- In addition to infectious disease diagnostics, antibody avidity measurements may be useful in other applications such as the determination of vaccine efficacy[5] and the characterization of autoimmune disorders.
- The use of Axela's panelPlus™ addressing reagents[6] allows for the immobilization of multiple capture molecules in a single sensor and may facilitate the development of multiplexed antibody titer and avidity assays.
- Real time, label-free observations of molecular interactions on the dotLab® mX System make it an excellent platform for reagent qualification, assay development and assay optimization[4].

References

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About Axela, Inc.

Axela's platforms provide powerful new approaches to multiplexed protein and nucleic acid analysis designed to greatly simplify biomarker testing in clinical research and diagnostics. Axela's commercial research products significantly improve the amount and quality of information derived from traditional assays. This approach shortens time to result and provides access to unique categories of markers that form a pipeline of future diagnostic offerings.

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