

Comparison of Antiserum Titers Using dotLab[®] System

Yixin Lin,
Axela Inc.,
Toronto, Canada

Jim Ritchie,
Emory University, Atlanta,
GA, United States

Background

An immunoassay measures the concentration of an analyte in a biological liquid using the reaction of an antibody to its antigen. The assay takes advantage of the specific binding of an antibody to its antigen. Antibody reagents are produced by injecting an antigen into a mammal (e.g., mouse, rat, goat, rabbit, donkey, sheep, horse) thereby producing polyclonal antibodies in the blood of the animal. The serum of such blood contains the antibodies and is called antiserum. With many sources of antibody available for research applications, an antibody's ability to bind with its antigen can be variable. Selecting an antibody with best binding for the antigen (i.e., a combined effect due to a higher antibody concentration, a higher affinity to the antigen, or both) is therefore desired before purification using protein A/G or antigen-affinity chromatography.

We demonstrate here an example of using the dotLab[®] System to quickly and easily compare the relative binding ability of antisera to an antigen. This binding measurement, which determines how one antiserum recognizes the antigen, is made to evaluate the titers. We compared the binding of two commercial antisera to corticotropin releasing factor (CRF) or hormone (CRH), a 5 kDa (41 a.a.) peptide. CRF is one of several neurohormones synthesized by specific hypothalamic nuclei in the brain and released into the portal system, which bathes the anterior pituitary. CRF also has marked CNS effects by acting at higher centers in the brain, particularly cortical regions where there is a widespread distribution of CRF neurons. The fundamental role of CRF is to prepare the organism for an appropriate response to various stressors such as physical trauma, insults to the immune system, and social interactions. It is the hyper- or hypo-sensitivity of the system that can lead to human pathologies such as anxiety, depression and eating disorders.

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 μ L flow channels forming a diffraction grating (Figure 1). The dotLab mX instrument illuminates the grating with a laser generating a diffraction image with 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 System simplifies and automates this analysis using a fully integrated, easy to use, bench top instrument.

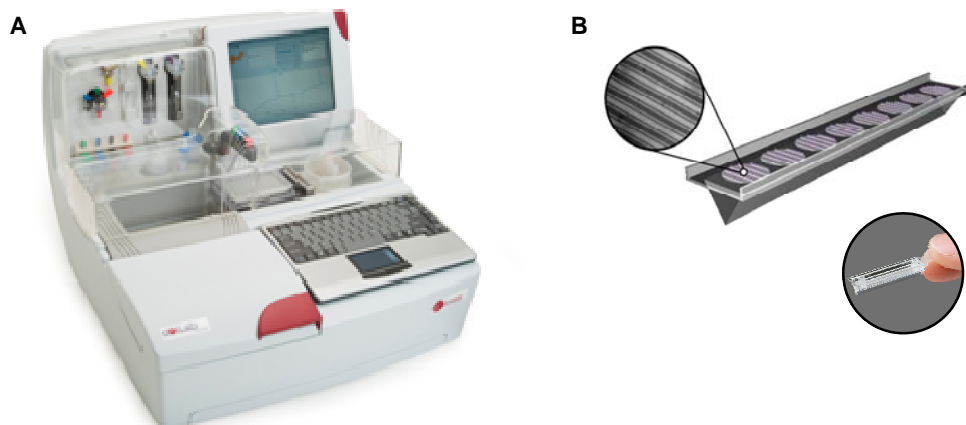
Materials

Anti-CRF (3-41): rabbit antiserum against pTG conjugated synthetic CRF (3-41) human, rat peptide (Cosmo Bio, Tokyo, Japan).

Anti-CRF (24-41): rabbit antiserum against a carrier free synthetic CRF (24-41) (human, mouse, rat) peptide (Cosmo Bio, Tokyo, Japan).

Biotinylated CRF (bt-CRF): Synthetic N-terminal biotinylated CRF (human, rat) peptide (5 kDa) (Bachem, Bubendorf, Switzerland).

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.



Methods

The assays were performed in dotLab® Sensors with avidin surface (Axela Inc.). All experiments were carried out on the dotLab System (Axela Inc.) and real-time traces were recorded accordingly.

ANTISERUM TITER COMPARISON FOR CRF

Running Buffer containing PBS (0.154 m NaCl, 0.01 m phosphate, pH 7.4) with 0.025% Tween-20 was introduced into a dry avidin sensor for 200 seconds to stabilize the flow system and remove the preservatives from the sensor. BSA (5 mg/mL of BSA in Running Buffer) was introduced and incubated for 5 minutes in mixing mode (by repeatedly reversing flow directions within the sensor). The mixing mode was used in all subsequent incubations. 10 µg/mL of biotinylated CRF diluted in BSA was introduced and incubated for 10 minutes. The sensor was washed with Running Buffer. BSA was introduced and incubated for 5 minutes. A dilution of antiserum 3-41 or 24-41 in BSA was introduced and incubated for 10 minutes. The sensor was washed at the end of run with Running Buffer.

DATA ANALYSIS

All data recorded in dotLab® Software were exported as a csv file and analyzed by GraphPad Prism® (GraphPad Software Inc., San Diego, CA).

Results and Discussion

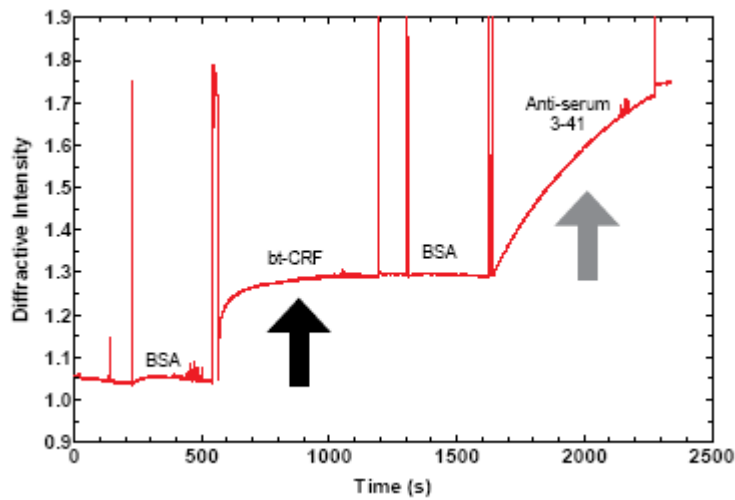
CAPTURE ANTISERUM 3-41 By BIOTINYLATED CRF

An antigen-down immunoassay (a dotReady™ immunoassay) was developed using dot® Technology to compare the binding of antisera to the antigen, CRF. For all assays, biotinylated CRF, a synthetic full-length CRF peptide with N-terminus biotinylated, was immobilized as capture molecules on a dotLab Sensor with patterned rows of avidin molecules on its surface. Bt-CRF captured specific antibodies from subsequently introduced antiserum samples. The immuno-capture resulted in the buildup of a complex on sensor surface and the increase of total complex sizes led to increased diffractive intensity (DI) signals that were recorded as real-time traces.

Figure 2A is a typical trace showing the capture of an antiserum by immobilized antigen. Bt-CRF was first immobilized on the avidin sensor surface through the avidin-biotin interactions and the binding event was observed in real time as an upward curve (black arrow). All the binding/blocking/washing events occurred in the sensor. A mixing mode (by repeatedly reversing flow within the sensor) was used to enhance interactions, much the same way as mixing a reaction by vortexing a tube or shaking a plate. The change in DI from the introduction of the bt-CRF to its removal corresponds to the binding of bt-CRF to the avidin surface. BSA blocking buffer was always introduced prior to loading of either a capture molecule or an analyte.

Figure 2A. Capture of antiserum 3-41 by bt-CRF using dotLab Technology.

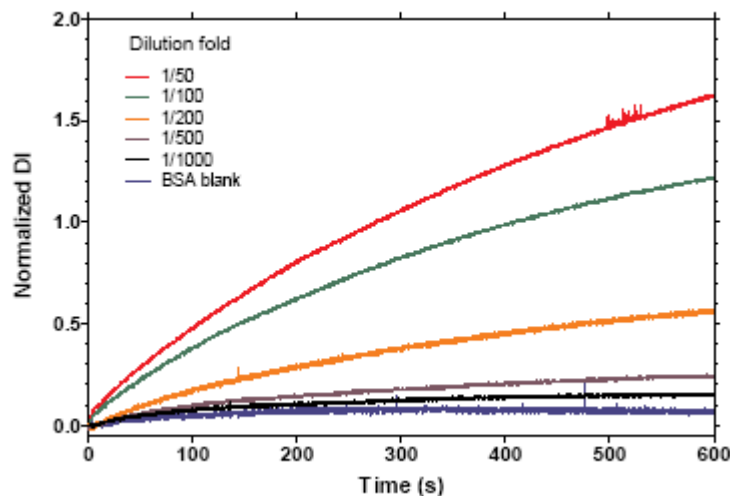
A real-time trace showing bt-CRF captured specific antibodies from antiserum 3-41. 10 µg/mL of bt-CRF was first immobilized on an avidin sensor, shown as an upward curve (black arrow). 1/50 dilution of antiserum 3-41 was introduced and specific antibodies were captured by bt-CRF (gray arrow). All non-labeled portions are Running Buffer (PBS with 0.025% Tween-20, pH 7.4) wash. BSA (5 mg/mL in Running Buffer) was introduced prior to the introduction of each reagent (bt-CRF and antiserum). All reagents were diluted in BSA. Spikes in DI tracing are air gaps separating each reagent introduction.



Antiserum 3-41 [1/50 (v/v) dilution in BSA] was introduced and the specific antibodies were captured by immobilized bt-CRF, as shown in the second upward curve (gray arrow). In the analyte negative control, where BSA (blank) instead of antiserum 3-41 was used as analyte, there was no capture by bt-CRF, resulting in a flat line (Figure 1B, blue trace). A series of dilutions of antiserum 3-41 in BSA, including 1/1000, 1/500, 1/200, 1/100, and 1/50 dilutions, were performed to compare their capture by bt-CRF. Their bindings were overlaid as shown in Figure 2B. The normalized DI, which is the ratio of DI signals of antiserum 3-41 to the maximum DI change of bt-CRF, was used to minimize inter-sensor variation. Because the binding profile of 1/50 dilution showed the best DI signals with ease of comparison, this condition was chosen to compare the relative binding ability of 3-41 and 24-41 to bt-CRF.

Figure 2B. Capture of anti-serum 3-41 by bt-CRF using dotLab Technology.

Overlay of the antibodies in antiserum 3-41 captured by bt-CRF when 3-41 was diluted 1/1000, 1/500, 1/200, 1/100, and 1/50 folds in BSA. The blue trace is BSA blank negative control where BSA instead of 3-41 was used as analyte. Note that "normalized DI" is the ratio of antiserum DI to the maximal DI change of the bt-CRF in each experiment.

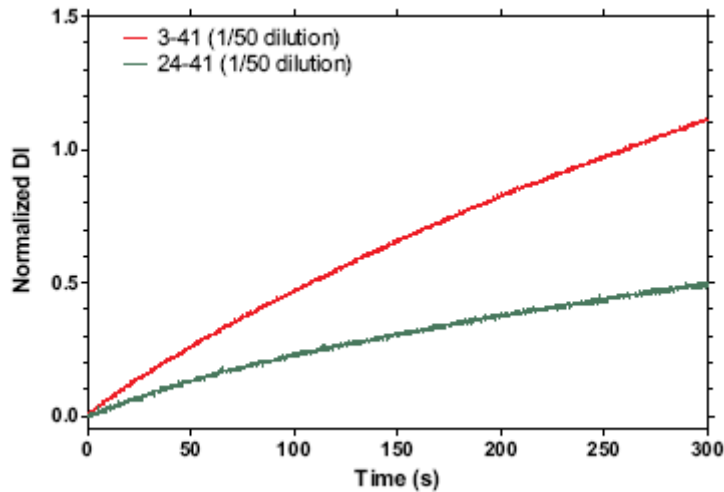


COMPARISON OF RELATIVE BINDING OF ANTISERA TO CRF

The normalized DI signals of antiserum 3-41 and antiserum 24-41 captured by bt-CRF were overlaid as shown in Figure 3. This showed that under the same experimental conditions, 3-41 had better binding ability than 24-41 to CRF.

Figure 3. Comparison of relative binding of antisera to CRF.

10 µg/mL of bt-CRF was immobilized to capture 1/50 diluted antisera. Only the normalized DI signals of antibodies captured by bt-CRF were shown.



Conclusion

In these experiments, the dotLab System ranked the relative binding ability of antiserum samples to the antigen providing a quick and easy method for the selection of antibodies for use in immunoassays. The 3-41 antiserum demonstrates higher titer than 24-41 for CRF. With a single binding experiment taking less than 30 minutes and inexpensive disposable sensors, the dotLab System provides a quick and easy way of comparing antiserum titers at very low cost.

dotLab® System, dotReady™ Reagents and associated software are for Research Use Only.

Not for Diagnostic Purposes.

- 50 Ronson Drive
- Suite 105
- Toronto, Ontario
- Canada M9W 1B3

Tel: 1 • 416 • 798 • 1625
 Fax: 1 • 416 • 798 • 8635

About Axela Inc.

Axela's platforms provide powerful new approaches to multiplexed DNA, RNA and protein 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.

www.axela.com

