WHITEPAPER

Development of a Rapid Serological Assay for Strongyloides Infection Using Diffraction-Based Optical Biosensors

Brian J. Pak[1], Fabio Vasquez-Camargo[2], Paul T. Smith[1], Momar Ndao[2]

[1]Axela, Inc. Toronto, Ontario, CANADA; [2]National Reference Centre for Parasitology, Montreal, Quebec, CANADA

Background

Strongyloidiasis is a persistent parasitic disease caused by the intestinal nematode *Strongyloides stercoralis*. It is endemic in the tropical and subtropical regions of the world with increasing prevalence in North America and Europe due to widespread travel and immigration. Most individuals with Strongyloidiasis are asymptomatic and unaware of infection. Moreover, due to its ability to internally autoinfect the host, Strongyloidiasis can persist for life. Immunosuppression in infected individuals can result in hyperinfection and disseminated disease with an associated mortality of over 80%[1]. Therefore, testing for Strongyloides infection, particularly in patients about to receive immunosuppressive therapy, has significant clinical utility.

Current methods of Strongyloides testing include stool culture and ELISA-based serology, both of which are labor intensive and time consuming. Stool culture assays suffer from poor sensitivity as the parasite is not consistently shed into the stool. ELISA analysis exhibit variable sensitivity and specificity depending on the antigen preparation used but generally show significant cross reactivity with other helminths infections.

The dotLab® mX System

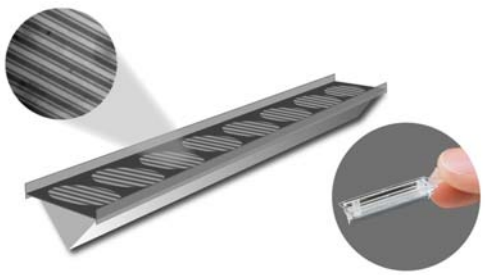
The dotLab® mX System utilizes diffraction-based optical sensing for the real time, label-free measurement of molecular interactions. The system uses inexpensive, disposable biosensors with coupling reagents (eg: avidin, amine reactive substrates or unique oligonucleotide-based addressing reagents to 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

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.

A

B



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.

axela

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 0.05% Tween 20, (PBST). Low Cross Buffer (Candor Bioscience GmbH) was used as blocking buffer and sample diluent. NIE recombinant antigen from *S. stercoralis* L3 stage larvae[2] was obtained from Dr. F.A. Neva (National Institutes of Health) and biotinylated using the EZ-Link Sulfo-NHS-LC Biotinylation Kit (Pierce) according to the manufacturer's instructions but using only one-third of the recommended amount of biotin. Anti-human IgG antibodies were obtained from Perkin Elmer.

dotLab Analysis

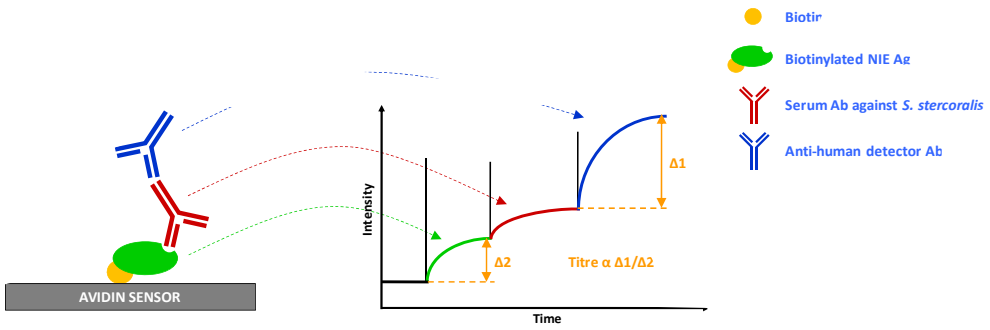
Biotinylated recombinant NIE antigen (5 µg/mL stock) was immobilized on avidin-coated dotLab sensors for 5 minutes with gentle agitation. Antibodies were captured from patient serum samples (diluted 10-fold in blocking buffer) yielding a binding curve proportionate to the antibody titer. Antibody binding signal was amplified using an anti-human IgG detection antibody. All reagent incubations and wash steps were automated following pre-programmed methods allowing for unattended operation.

Data Analysis

The amplitude of the anti-human detection antibody binding signal was normalized to the amplitude of biotinylated NIE antigen binding was used as a measure of Strongyloides antibody titer (Figure 2).

Figure 2

Schematic of the dotLab-based Strongyloides serological assay.



Results

Figure 3A shows a representative trace obtained from a Strongyloides positive patient. The binding of biotinylated NIE, serum Strongyloides antibodies and anti-human IgG detection antibodies can each be seen binding directly to the sensor. Total assay time was less than 40 minutes. Panel B shows the detection antibody binding portion of the assay for samples obtained from healthy, Schistoma and Trichinella patients.

Figure 3

(A) Representative trace of a dotLab Strongyloides assay. (B) Anti-human detection antibody amplified signal for Strongyloides positive and control samples.

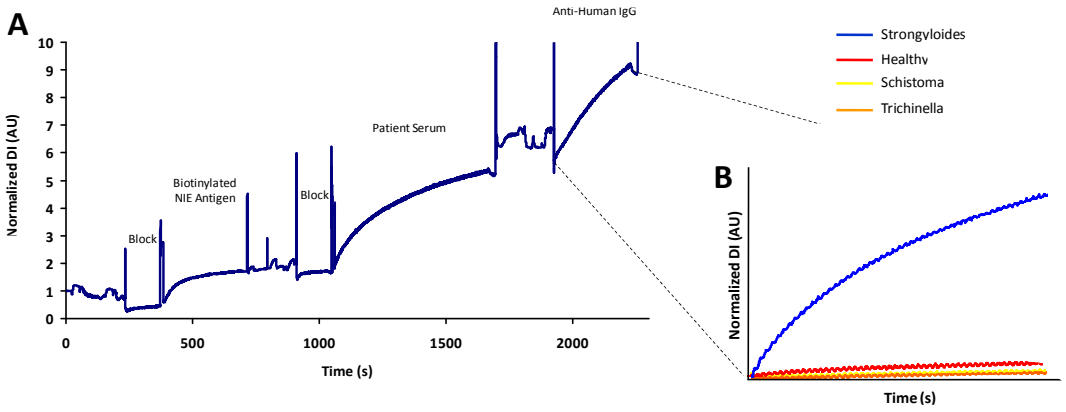
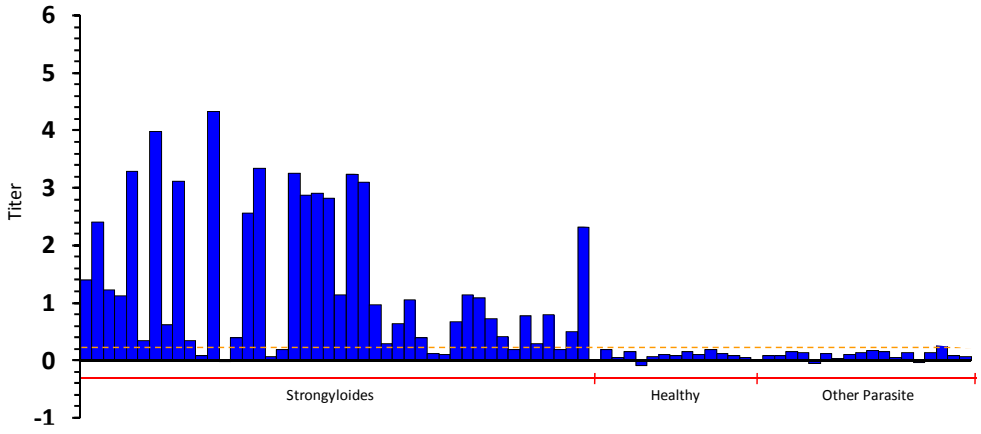


Figure 4 summarizes the test results of 75 individual samples comprised of Strongyloides positive patients, healthy and individuals with other parasitic infections. Overall, this assay performed with a sensitivity of 82% and specificity of 97%. These results also showed an 88% agreement with NIE ELISAs.

Figure 4

Titer results for 75 patient serum samples from the following groups: 1) Strongyloides positive; 2) healthy; and 3) other parasitic infections. The dotted line represents the threshold above which samples are considered Strongyloides positive.



Summary and Conclusions

A rapid and sensitive serology assay was developed for the determination of Strongyloides infection which can be performed in less than 40 minutes.
On a set of 75 patient serum samples, the assay performed with a sensitivity of 82% and a specificity of 97%.
Multiplexed serological assays can be performed on single patient samples using Axela's panelPlus™ addressing reagents[3] which allows for the immobilization of multiple antigens on a single sensor.
The real time, label free detection of molecular interactions on the dotLab System makes it an excellent platform for rapid reagent qualification, assay develop and assay optimization.[4]

References

1. Ingra-Siegman *et al.* (1981) Syndrome of hyperinfection with *Strongyloides stercoralis*. Rev. Infect. Dis. 3:397-407.
2. Ravi *et al.* (2002) Characterization of a recombinant immunodiagnostic antigen (NIE) from *Strongyloides stercoralis* L3-stage larvae. Mol. Biochem. Parasitol. 125:73-81.
3. Lin *et al.* (2009) Intra-feature and inter-feature multiplexing using diffractive optics technology: more information from less sample. Presented at the 41st annual Oak Ridge conference.
4. Chen *et al.* (2007) Accelerating the immunoassay process: from development to implementation. Presented at Clinical Ligand and Assay Society annual meeting.

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.

For research use only.

axela

50 Ronson Dr, Suite 105
Toronto, Ontario
Canada
M9W 1B3

Ph: 1.866.94.AXELA (toll free)
or +1.416.798.1625
Fax: +1.416.798.8635

www.axela.com
info@axela.com