

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

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Background

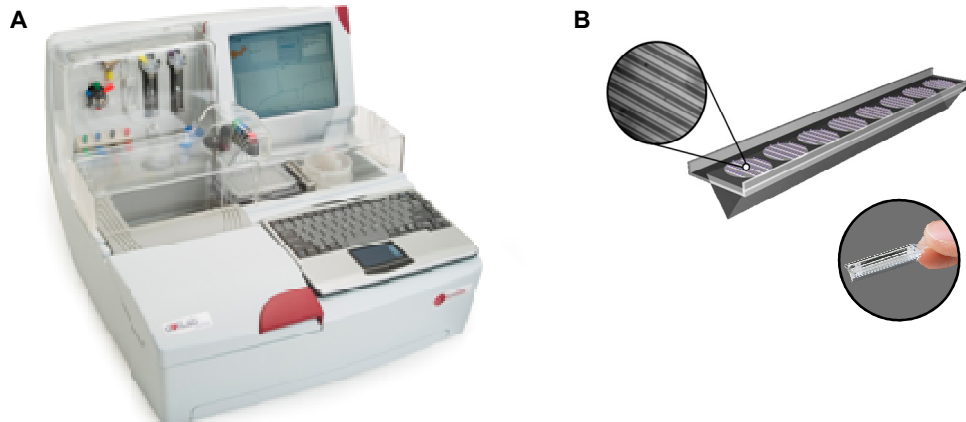
Strongyloidiasis is a persistent parasitic disease caused by the intestinal nematode *Strongyloides stercoralis*. It is endemic in the tropical and subtropical regions of the world with increasing prevalence in North America and Europe due to widespread travel and immigration. Most individuals with Strongyloidiasis 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 for multiplexing) pre-patterned on the surface of 10 μ L flow channels forming a diffraction grating (Figure 1). The dotLab mX instrument illuminates the pre-patterned 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.



with a laser generating a diffraction image which is monitored by a photodiode detector. Diffractive 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 diffractive 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, bench top instrument.

Here, we demonstrate the use of the dotLab mX System to develop and perform rapid serological analysis of *Strongyloides* infection.

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 larvae² 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.

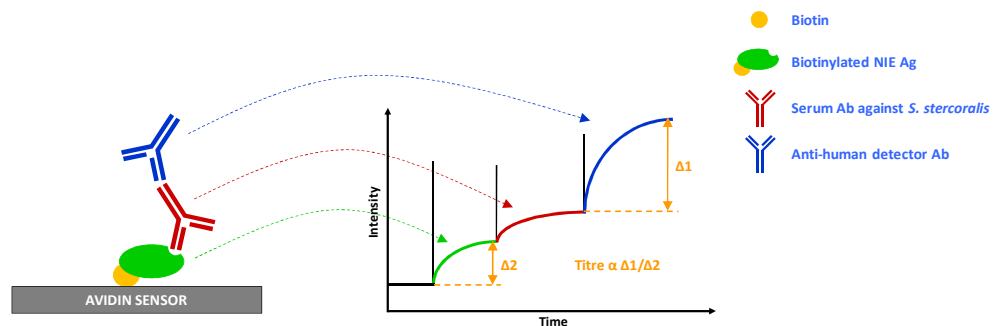
dotLab Analysis

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.

