

Rapid Determination Of Strongyloides Infection Using A Novel Diffractive Optics Technology

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Abstract

Strongyloidiasis, caused by the intestinal nematode, *Strongyloides stercoralis*, is endemic in tropical and subtropical regions of the world with increasing prevalence in North America. Infected individuals are typically asymptomatic. However, when immunocompromised, the infection can rapidly progress to a hyperinfected and disseminated state, with an associated mortality of approximately 90%. Therefore, a rapid means of diagnosing Strongyloides infection may significantly reduce mortality. Current diagnostic serological tests use a crude Strongyloides extract to detect serum antibodies. This approach is time consuming and prone to false positive results due to the cross reactivity of components of the extract to antibodies from related parasitic infections. Here, we describe a rapid serological method of determining Strongyloides infection. The method involves the use of a recombinant Strongyloides antigen to capture antibodies from only 10 µl of serum. Antibody binding was measured using the dotLab[®] System, a novel, automated technology which monitors the real-time interaction of proteins by diffraction-based optical sensing. This assay successfully detected serum Strongyloides antibodies in approximately 30 minutes and showed almost no cross reactivity to sera from healthy individuals or patients with other parasitic infections. In addition to general Strongyloides testing, this assay may have potential use in acute, point of care settings where rapid diagnosis is needed.

Diffractive-Based Optical Sensing

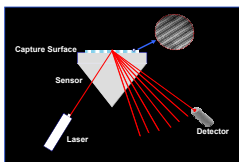


Figure 1. Principles of diffractive-based optical sensing.

Diffractive-based optical sensing is based on two basic principles: grating-based light diffraction and immobilized affinity surfaces. This combination produces a sensitive and rapid technique for the detection of biomolecular interactions without the use of fluorescent or chemiluminescent labels. Capture molecules are immobilized in a specific pre-patterned series of repeating parallel lines which when illuminated by a laser light generates a consistent diffraction image. Subsequent binding of molecules to the patterned surface results in an increase in the intensity of the diffraction image, yielding a real-time measurement of the binding event. Conversely, the dissociation of molecules from the capture surface leads to a measurable decrease in signal intensity.

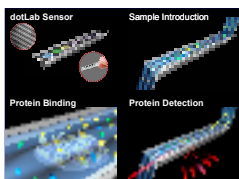


Figure 2. Detection of biomolecular interactions using the dotLab[®] System.

The dotLab System utilizes disposable biosensors which consist of a series of pre-patterned affinity surfaces on the bottom of a 10 µl flow channel. Biological samples are introduced into the flow channel by the instrument's fluidic system following user defined methods. As proteins bind to the surface of the sensor, the surface is interrogated by a laser, yielding real-time protein interaction data. An optical prism positioned below the flow channel reflects the incoming laser beam and the resulting diffraction pattern. Therefore, the beam does not pass through the flow channel thus minimizing the effects of changes in sample refractive indices on signal baseline, making the dotLab System ideal for working directly with crude biological samples.

Materials and Methods

Samples:

- serum samples were obtained from three patient groups:
- Group I: Stool positive for *S. stercoralis* larvae
- Group II: Healthy individuals
- Group III: Positive for other parasitic infections including Trichinella, Filaria, Echinococcus, Schistosoma and Hookworm

Reagents:

- NIE recombinant antigen for immunodiagnosis of human Strongyloides infection was obtained from Dr. F.A. Neva (NIH)
- NIE biotinylation was performed using the EZ-Link Sulfo-NHS-LC Biotinylation Kit (Pierce) according to manufacturer's instructions but using only one-third of the recommended amount of biotin
- anti-human IgG1, IgG4 and IgE antibodies were purchased from BD Biosciences
- anti-human IgG3 antibodies were purchased from AbD Serotec
- avidin biosensors and all other reagents were obtained from Axela, Inc.

Diffractive-Based dotLab[™] Serological Assay:

- all serological assays were performed using the dotLab[™] System (Axela, Inc)
- assays were conducted in a running buffer of PBS containing 0.05% Tween-20 (PBST)
- sensors were blocked with 5 mg/ml BSA prior to the immobilization of biotinylated NIE antigen (5 µg/ml)
- sensors were further blocked with normal human serum diluted 1:10 in PBST, immediately followed by the addition of 10 µl of patient serum diluted 1:10 in PBST
- for antibody isotyping, each anti-isotype antibody was sequentially applied at a concentration of 10 µg/ml with a PBST wash and BSA block between each application

Data Analysis:

- data files were exported from the dotLab Software in comma separated value (csv) format and imported into Microsoft[®] Excel
- the amplitude of antibody binding signal following patient serum incubation was normalized to the amplitude of NIE antigen binding to yield a DI ratio which was used as a measure of serum Strongyloides antibody levels



Figure 3. Schematic representation of NIE capture of serum antibodies against *S. stercoralis* on avidin biosensors

Results

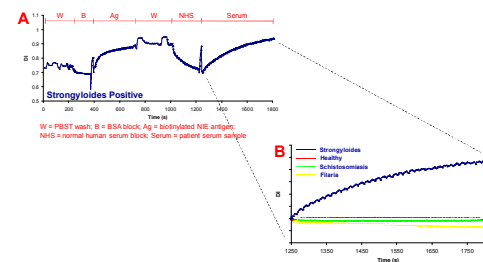


Figure 4. Representative result from the dotLab-based Strongyloides serological assay: Panel A shows a typical trace obtained from the analysis of a Strongyloides positive patient. Note the detection of Strongyloides antibody binding when patient serum is applied. Panel B shows representative results obtained from the analysis of sera from healthy individuals (red), Schistosomiasis positive (green) and filaria (yellow) patients. Total assay time was approximately 30 minutes.

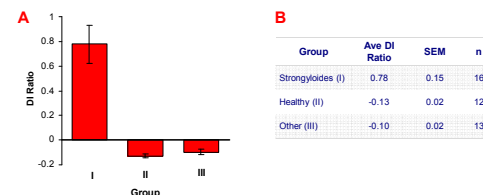
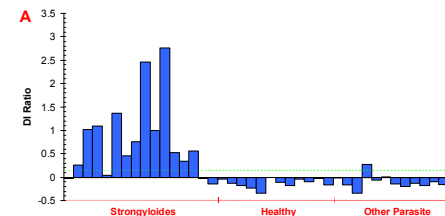


Figure 5. A total of 42 patient samples consisting of (I) 17 Strongyloides positive, (II) 12 healthy individuals and (III) 13 with other parasitic infections were analysed for the levels of serum antibodies against NIE. Panels A and B summarize the average normalized signal from serum *S. stercoralis* antibody binding to NIE obtained using the dotLab System. Data represent mean ± SEM.



dotLab-based Serology Results	
Strongyloides (n=16)	12 Positive
Healthy (n=12)	0 Positive
Other Parasite (n=13)	1 Positive
Sensitivity	75%
Specificity	96%

Figure 6. dotLab-based Strongyloides serological test results using NIE recombinant antigen. Panel A shows the individual normalized Strongyloides antibody binding signal obtained by the dotLab System for each sample in the study. The green dotted line indicates the positive threshold above which samples are considered Strongyloides positive. The positive threshold level was calculated as the average normalized signal from all healthy and other infection samples + 2 standard deviations. Panel B summarizes the number of positive results obtained from each clinical group. The dotLab-based Strongyloides serological test using NIE recombinant protein yielded a sensitivity of 75% and specificity of 96%.

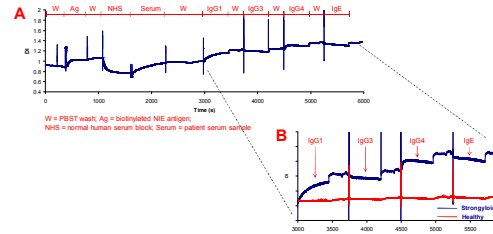


Figure 7. Strongyloides antibody isotyping. Panel A summarizes the complete analysis of a Strongyloides positive serum, which includes the detection of serum Strongyloides antibodies using recombinant NIE and the sequential probing with anti-human IgG1, IgG3, IgG4 and IgE antibodies to generate an isotype profile all in a single assay. Panel B shows the isotyping portion of the trace for a Strongyloides positive and healthy individual. Note the detection of IgG1 and IgG4, and the absence of IgG3 and IgE in this Strongyloides patient and the absence of all four antibody isotypes in the healthy individual.

Summary and Conclusions

• A Strongyloides serological test was developed using NIE recombinant protein on the dotLab System. Preliminary results based on a limited number of samples analysed showed an assay sensitivity of 75% and specificity of 96%.

• Test results were obtained in approximately 30 minutes, making this dotLab-based Strongyloides serological test conducive to possible future point-of-care applications where rapid results are required.

• Sequential probing of Strongyloides positive samples with isotype antibodies enabled the determination of antibody isotype profiles in infected patients in a single assay. Isotype profiles may improve serodiagnosis and may provide information on disease status such as chronicity or drug resistance.



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