WHITEPAPER

Direct Pathogen Detection by Diffractive Optics Technology

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Abstract

Direct pathogen detection by diffractive optics technology was demonstrated using the dotLab® System on a variety of crude samples infected with either bacterial or viral pathogens. These immunoassays used real time diffraction monitoring to detect the binding of intact pathogens on the surface of diffractive optics sensor. Using the sequential addition of reagents to the dotLab® System and, in many cases, various signal amplification techniques, sensitive assays were achieved for influenza A (H1N1) and norovirus from nasal swabs and stool samples respectively, as well as for *E. coli* and *S. epidermidis* in spinach extracts and urine samples. Overall, these assays illustrate the potential for diffractive optics technology as an alternative to traditional pathogen detection techniques that are currently too laborious and time consuming to be performed outside of specialized laboratories.

Introduction

The ability to detect intact viral and bacterial pathogens from crude samples has significant applications both in food and environmental analysis and in clinical diagnostics. Most current methods for pathogen detection require culturing the pathogen to increase their number and amplifying its genome by polymerase chain reaction (PCR). These methods are labor intensive, expensive and often need to be performed by highly skilled personnel working in specialized laboratory facilities. Therefore, there is a need for rapid and easy to use assays for infectious disease pathogens in order to accelerate the treatment of infected patients and to locate the source of contamination in the environment to control outbreaks.

The use of diffractive optics technology is well suited for the detection of unicellular pathogens due to the relatively large sizes of the analytes being assayed. Combined with the fluidics technology of the dotLab® System, it is also capable of detecting pathogens in crude samples, eliminating the need for laborious sample preparations and thus delays in obtaining critical results. To demonstrate the use of diffractive optics in pathogen detection, a series of real time immunoassays were developed on the dotLab® System on a variety of crude samples containing clinically relevant viral and bacterial pathogens. Herein, we describe viral assays for influenza A (H1N1) and norovirus from nasal swabs and stool samples respectively, as well as bacterial assays for *E. coli* and *S. epidermidis* in spinach extracts and urine samples. These results illustrate the potential use of diffractive optic technology for rapid point of use tests for infectious diseases or for the analysis of food and environmental samples.

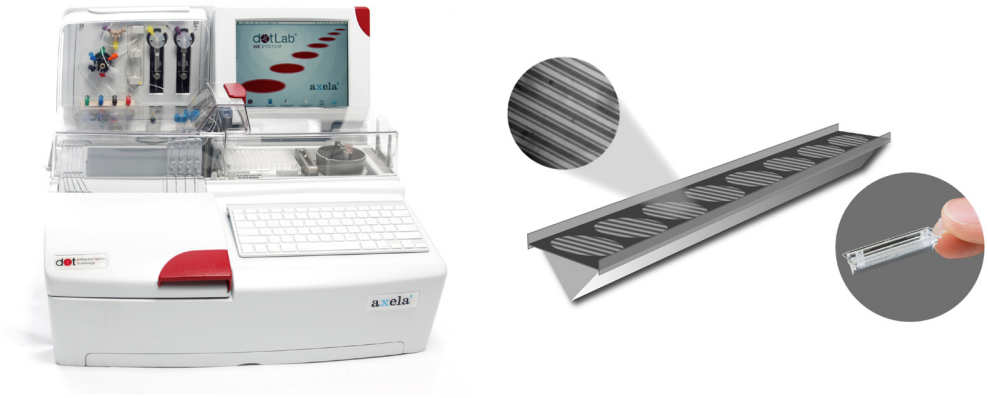
The dotLab® mX System

The dotLab® mX System utilizes diffraction-based optical sensing for the real time, label-free measurement of molecular interactions. The system uses 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 diffraction grating is illuminated with a laser generating a diffraction image, the intensity of which is monitored by a photodiode detector. Diffractional efficiency increases as analytes bind to the sensor's surface resulting in an increase in image intensity, while molecular dissociation from the surface results in a decrease in image intensity. Large sized analytes, such as high molecular weight biomolecules or fully intact pathogens, also generally produce a higher intensity diffraction image compared to smaller sized analytes. The dotLab® mX System simplifies and automates diffractive optics technology by providing users a fully integrated, easy to use, bench top instrument for the analysis of complex molecular interactions.

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



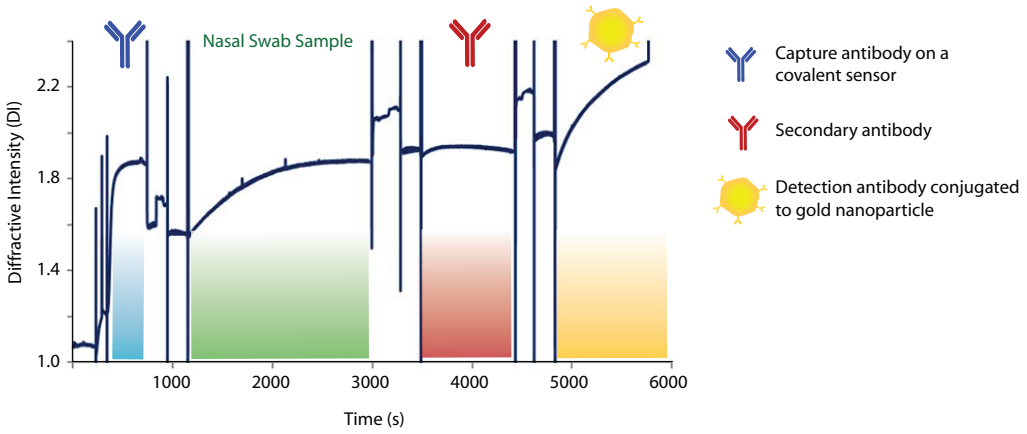
Detection of Influenza A Virus (H1N1) in Nasal Swabs

Rapid diagnosis and effective treatment of pandemic influenza infections are essential for preventing severe outbreaks and controlling the spread of disease. Although existing immunoassays for the influenza viruses are generally rapid, they often provide low sensitivity (10 to 70%) compared to the more labor intensive viral culture or RT-PCR techniques.[1] In an effort to develop a more sensitive and easy to perform influenza assay, direct pathogen detection by diffractive optics technology was investigated using the dotLab® System and nasal swab samples containing the influenza A virus.

In a typical assay, a capture antibody was immobilized onto an amine reactive sensor and incubated with a dilute nasal swab sample containing influenza A. The binding signal by the virus was enhanced by the sequential addition of a secondary antibody followed by a detection antibody conjugated to a 10 nm gold nanoparticle (Figure 2). In addition to providing a means of signal amplification, the secondary antibody provides also increases assay specificity and enables the determination of influenza substrains. Furthermore, by using multiplex panelPlus™ Sensors on the dotLab® mX System, multiple viral strains can be detected simultaneously thereby providing a means of combining diagnostic tests for different influenza virus types (A versus B) and subtypes (eg. novel influenza A H1N1 versus seasonal influenza A viruses).

Figure 2

Detection of influenza A virus in nasal swabs.



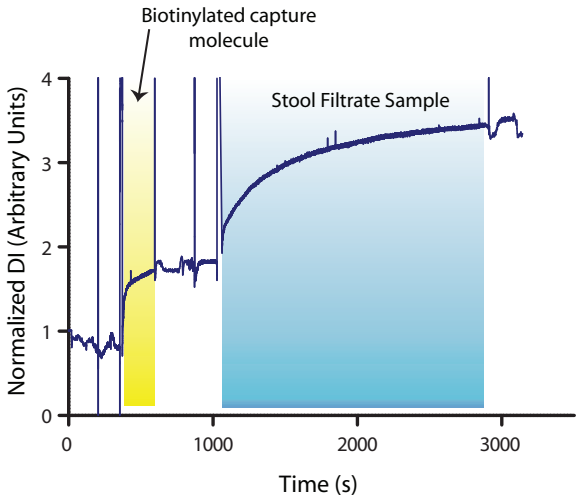
Detection of Norovirus in Stool Filtrate

Norovirus is a highly contagious virus that is recognized to cause up to 58% of food-borne illnesses each year in the United States.[2] It is primarily transmitted through a fecal-oral route, either by consuming fecally contaminated food or water, or by person to person spread.[3] Using the dotLab® System, a real time norovirus immunoassay was developed towards detecting the whole virus in stool filtrates.

In a model assay, a biotinylated capture molecule, known to bind specific receptors on the virus, was immobilized onto an avidin sensor and then incubated with undiluted stool filtrate from an infected patient. Real time binding of the norovirus to the sensor was observed in an assay that took less than one hour to complete (Figure 3). While signal amplification techniques as described above are capable of detecting viruses in very low titres, the unique flow through feature of the dotLab® System can allow for the continuous flow of a sample over the sensor surface. This process would result in an accumulation of viral particles on the sensor surface thereby increasing the detection of the virus in low titres or in high volume samples.

Figure 3

Detection of norovirus from stool filtrate.



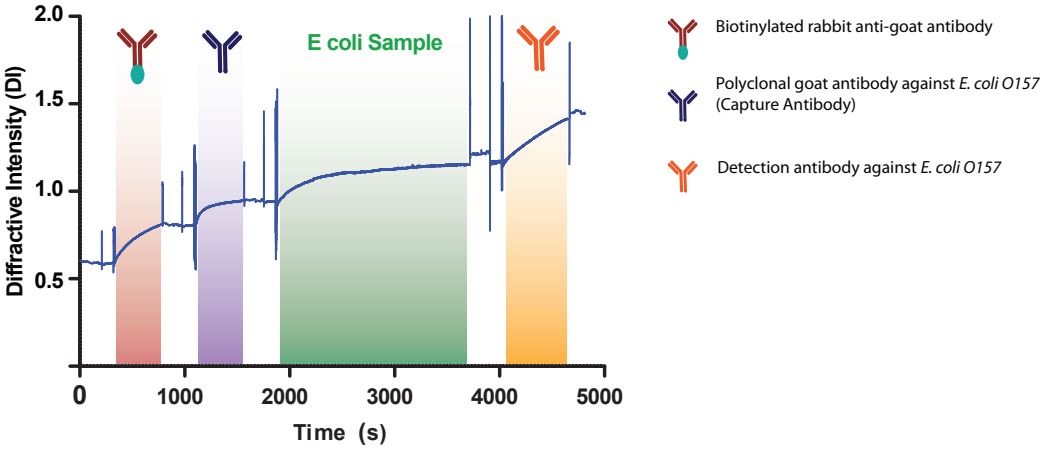
Escherichia coli O157 Detection

Shiga toxin-producing E. coli (STEC) exists in the guts of ruminant animals and is often acquired by ingesting food and water contaminated with very small amounts of human or animal feces. Rapid and accurate diagnosis of early stage STEC infection is vital to decrease the risk of renal damage in infected patients.[4] Prompt identification of the source of infection also assists in the prevention and control of outbreaks.

To investigate the use of diffractive optics as a platform for *E. coli* detection, samples containing the bacteria were analyzed in a sandwich immunoassay on the dotLab® System. Starting from an avidin coated sensor biotinylated rabbit anti-goat antibody was initially immobilized onto the sensor surface and used to capture a polyclonal goat antibody against *E. coli O157*. The bacteria sample in phosphate-saline buffer (PBS) was then applied followed by a detection antibody against *E. coli O157* (Figure 4). Similarly, a negative control containing *E. coli O145* was analyzed and gave no signal after addition of the detection antibody (data not shown). Each assay was completed in less than 90 minutes.

Figure 4

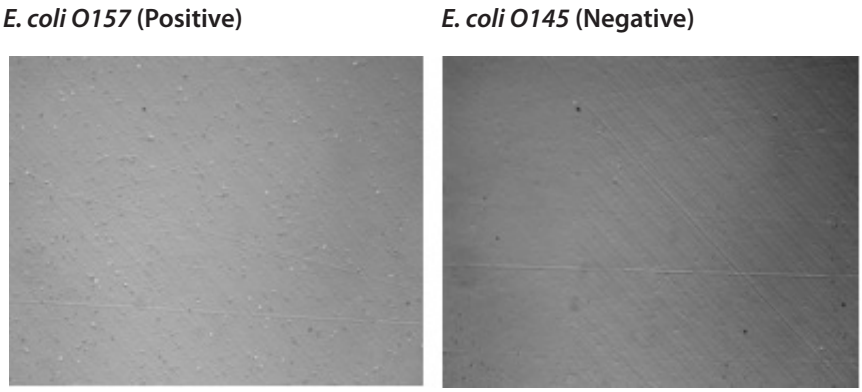
Detection of *E. coli* in PBS buffer.



Sensor surfaces were also viewed under a light microscope after completion of the *E coli* assays in PBS buffer. These images revealed the accumulation of *E. coli O157* primarily along the avidin coated diffraction lines rather then randomly throughout the sensor due to nonspecific interactions with the sensor surface. Images of negative controls using samples containing *E. coli O145* showed a clean sensor surface (Figure 5).

Figure 5

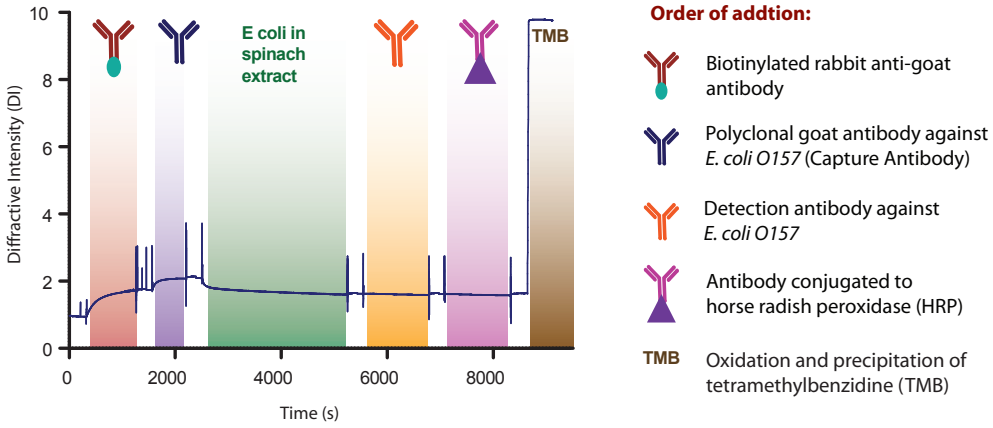
Light microscopic image of *E. coli* captured on a sensor surface.



To intensify the binding signal of bacteria and improve sensitivity of samples with low titres, various signal amplification techniques can be employed by the sequential addition of amplification reagents. In one set of examples, *E. coli O157* in spinach extract was analyzed on the dotLab® System using 3,3′,5,5′-tetramethyl-benzidine (TMB) oxidation-precipitation reactions at the end of the assays. For these assays, spinach extract was applied to the sensor-bound capture antibody as described above, followed by the detection antibody, and then a horse radish peroxidase (HRP) conjugated antibody before the addition of TMB (Figure 6).

Figure 6

Detection of *E. coli* in spinach extract by diffractive optics using TMB amplification.



Staphlococcus epidermidis Detection in Urine

S. epidermidis is a major cause of hospital acquired infections, and poses a significant risk to patients with immune deficiencies as well as people with catheters or other surgical implants due to its tendency to form biofilms on these devices. Since the bacteria are often resistant to commonly used antibiotics, the detection of *S. epidermidis* in infected patients is often critical for influencing their treatment options.

In a proof of principle study, *S. epidermidis* in urine was analyzed on a dotLab® System using an avidin coated optical sensor. To capture the bacteria, a biotinylated capture antibody was first immobilized onto the sensor. A urine sample containing *S. epidermidis* and a monoclonal antibody against Gram positive bacteria were pre-mixed in an Eppendorf tube and then applied to the sensor. The binding of the antibody/*S. epidermidis* complex was directly observed during real time monitoring of the assay (Figure 7). In a negative control assay, incubation of the monoclonal antibody without the bacteria onto the sensor yielded a no binding curve (data not shown).

A scanning electron microscope (SEM) image of the sensor spots following the *S. epidermidis* urine assay revealed numerous bacterial particles sitting on the diffraction grating of the avidin sensor after the addition of the monoclonal antibody and *S. epidermidis* to the capture antibody. A control using *S. epidermidis* in urine in the absence of the monoclonal antibody showed a mainly clean sensor surface (Figure 8).

Figure 7

Detection of S. epidermidis in urine.

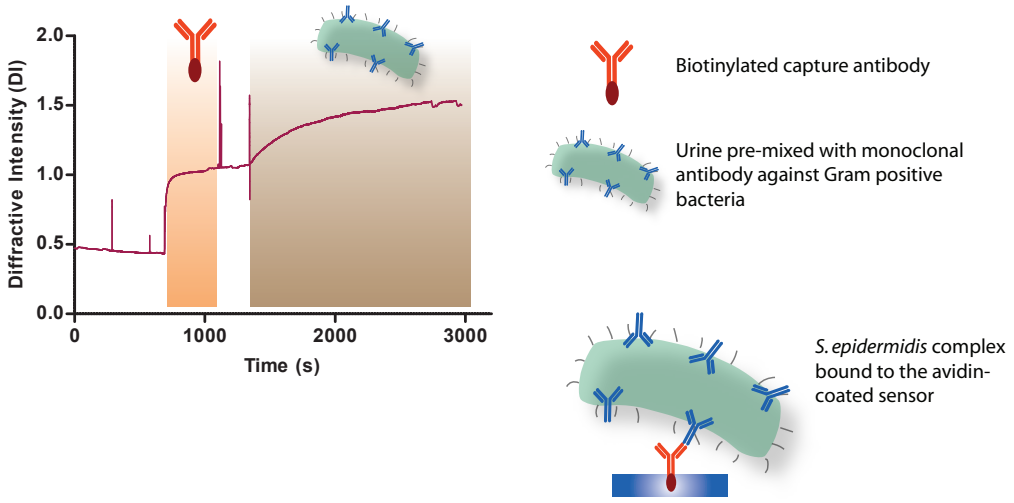
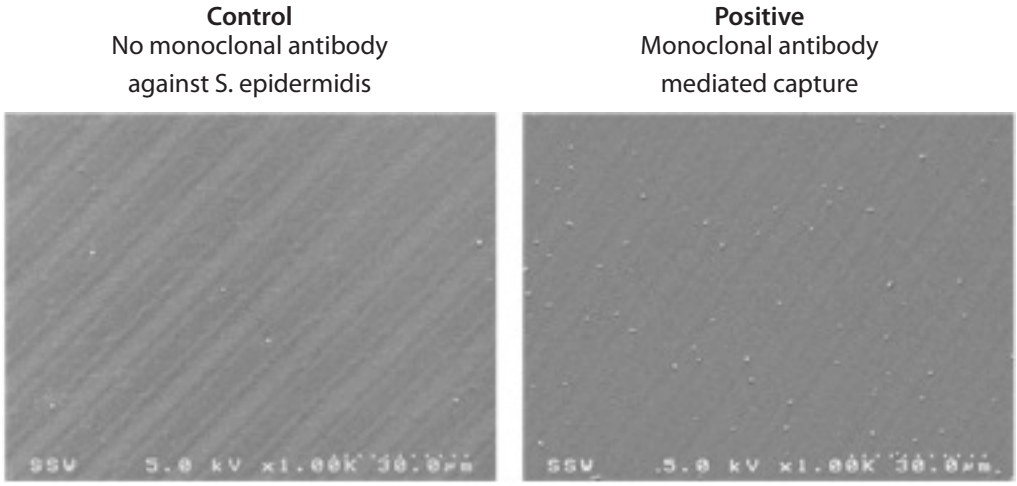


Figure 8

Scanning electron microscopic image of S. epidermidis captured on a sensor surface.



Conclusions

- The relatively large sizes of bacteria and viruses make them ideal for direct detection by diffractive optics technology and analysis by real time immunoassays on the dotLab® mX System
- A variety of biological, food and environmental samples can be employed with only minimal sample preparation thereby reducing the amount of labor and eliminating the need for pre-processing and pre-culturing by skilled personnel
- Rapid and sensitive real time assays can be optimized to obtain results in one hour or less and can include various signal amplification techniques for samples with low bacterial or viral titers
- Multiple strains can be analyzed in a single multiplex assay using Axela's panelPlus™ technology

References

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About Axela Inc.

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

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