

Spotted long oligonucleotide arrays for human gene expression analysis. *Genome Res* 2003;13:1775–85.

16. Park PJ, Cao YA, Lee SY, Kim JW, Chang MS, Hart R, et al. Current issues for DNA microarrays: platform comparison, double linear amplification, and universal RNA reference. *J Biotechnol* 2004;112:225–45.
17. Novoradovskaya N, Whitfield ML, Basehore LS, Novoradovsky A, Pesich R, Usary J, et al. Universal reference RNA as a standard for microarray experiments. *BMC Genomics* 2004;5:20.

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Diffraction Optics Technology: A Novel Detection Technology for Immunoassays, *Vitali Borisenko, Wei Hu, Pui Tam, Irene Chen, Jean-François Houle* and Walter Ausserer* (Axela Biosensors Inc., Toronto, Ontario, Canada; * address correspondence to this author at: Axela Biosensors Inc., 480 University Avenue, Suite 910, Toronto, Ontario, M5G 1V2, Canada; fax 416-260-9255; e-mail jf.houle@axelabiosensors.com)

There is an increasing need for high-sensitivity immunoassays that can be used in point-of-care patient testing of complex media. For example, analytes such as the natriuretic peptides and recently discovered sepsis markers are found in blood in very low picomolar concentrations (1, 2). Although advances have been made in the use of fluorescent, chemiluminescent, and other labels to measure markers at lower detection limits, background interference from biological samples and detection instrumentation remains problematic. Optical biosensors offer the promise of label-free real-time measurements, but their application to quantification of analytes in complex media is impaired by higher detection limits and is susceptible to changes in refractive indices or nonspecific surface binding. Moreover, the costliness of these devices has largely prohibited bedside implementation. In this report, we detail the use of a novel diffraction optics technology (dot™) that takes advantage of the inherent properties of diffraction optics to deliver a cost-effective, portable, robust, optical biosensor that detects analytes at picomolar concentrations in complex media.

In the dotLab™ System, coherent light striking a non-random pattern of capture molecules on the dotLab Sensor creates constructive and destructive interferences that produce a well-defined diffraction image. As molecules bind to the capture molecules, the height of the diffraction pattern is increased, which in turn increases the diffraction efficiency and the diffractive order intensity. A photodiode monitors the intensity of the diffractive order, which is correlated to analyte concentrations. Because diffraction is inherently self-referencing, the transduction of binding events is dependent on the initial pattern, and an increase in diffractive order intensity will occur only if molecules bind exclusively to the patterned capture reagents. Therefore, nonspecific binding to both

the patterned and nonpatterned regions will not affect the signal, a characteristic that offers an important advantage over other optical biosensor systems in which any surface-binding event will cause an increase in signal.

Previous diffraction-based immunosensors have used silicon wafer chips that were in contact with analyte-containing solutions and required washing and drying before analysis by a simple reader (3). The dotLab system uses a plastic consumable, the dotLab Sensor, with an integrated prism situated below the flow channel so that the light source interrogates the diffraction grating without passing through the bulk solution. Previous studies suggest that this total internal reflection scheme allows for 95% of the laser intensity to be measured, whereas in a nontotal internal reflection set-up only 5% is measured (4). Moreover, with this configuration, our system monitors biomolecule binding in real time and in complex media.

We tested the beta prototype of the dotLab instrument, which incorporates the core dot technology in an integrated package intended for in-house and controlled external development work. The instrument includes precision fluidic control of reagents, buffers, and samples—a proprietary integrated optical assembly designed to function with the dotLab Sensor; and software for acquisition, control, and user-interface. A movable stage allows monitoring and potential patterning with different capture reagents for 8 discrete diffraction spots. In addition, each individual spot can be cross-patterned to perform intraspot multiplexing and assays, as we have previously demonstrated (5, 6).

We used a single-spot and a streptavidin-patterned sensor with reagents for the detection of N-terminal probrain natriuretic peptide (NT-proBNP) in various matrices at reference concentrations, as summarized in Table 1. For direct detection of recombinant NT-proBNP, we used a biotinylated monoclonal antibody directed against NT-proBNP immobilized on a streptavidin-patterned dotLab Sensor (Fig. 1A). The binding event can be observed in real time in the trace. The binding of recombinant NT-proBNP is also detected, but at a lower intensity. The recombinant protein has a low relative molecular mass (M_r) of ~8000, which generates very little signal on its own. At lower concentrations, we used a different format to enhance the diffraction signal. For example, to detect NT-proBNP at nanomolar concentrations, we used a combination of capture and detector antibodies (Fig. 1B), with which the binding of the biotinylated mouse capture antibody was readily detected, whereas the introduction of the recombinant protein did not produce a detectable signal increase.

We carried out rinse steps with pulses of phosphate buffered saline (PBS) (137 mmol/L NaCl; 2.7 mmol/L KCl; 10 mmol/L $\text{Na}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$, pH 7.4; OmniPur) and PBS-Tween (10 mmol/L phosphate buffer, pH 7.4; 140 mmol/L NaCl; 3 mmol/L KCl; 0.025% (w/v) Tween-20; Calbiochem). When we introduced a polyclonal goat antibody directed against NT-proBNP, the diffraction signal increased. For internal calibration purposes and to

Table 1. Experimental components and assay format.

Concentration μmol/L	Matrix assayed	Reagent addition	Capture reagent	Detector reagent	Amplification reagent	TMB
	PBS	Sequential	Biotinylated mouse monoclonal anti-NT-proBNP	N/A ^a	N/A	N/A
nmol/L	PBS	Sequential	Biotinylated mouse monoclonal anti-NT-proBNP	Polyclonal goat anti-NTproBNP	N/A	N/A
pmol/L	NT-proBNP affinity stripped plasma-EDTA	Mixture-TMB post wash	Biotinylated mouse monoclonal anti-NT-proBNP	Polyclonal goat anti-NTproBNP	Antigoat HRP-conjugated donkey	1C TMB

^a N/A, not applicable

minimize any impact of intersensor variability, we calculated the ratio of the signal increase for the detector-binding event to that for the capture-antibody binding event. With this approach we generated a calibration curve for recombinant NT-proBNP spanning 10 to 1000 μg/L, or ~1.25 to 125 nmol/L in a PBS matrix (Fig. 1C).

For detection at picomolar concentrations, we added a 3rd antibody, donkey antigoat horseradish peroxidase conjugate, to the assay format. To investigate the compatibility of this system with a typical clinical matrix, we ran these assays in a human plasma matrix stripped of

endogenous NT-proBNP. For these experiments, we incubated the recombinant NT-proBNP with all 3 antibodies (see Table 1) in a single mixture before loading onto the sensor surface. The 90-min preincubation was necessary because of the relatively low on-rate of 1 antibody in the mixture. The binding event detected after loading the mixture reflects the binding of free and complexed biotinylated monoclonal antibody. After being washed briefly in both PBS-Tween and PBS, the complexes were detected by incubation with a precipitating form of 3,3',5,5' tetramethylbenzidine (TMB) [1-component TMB

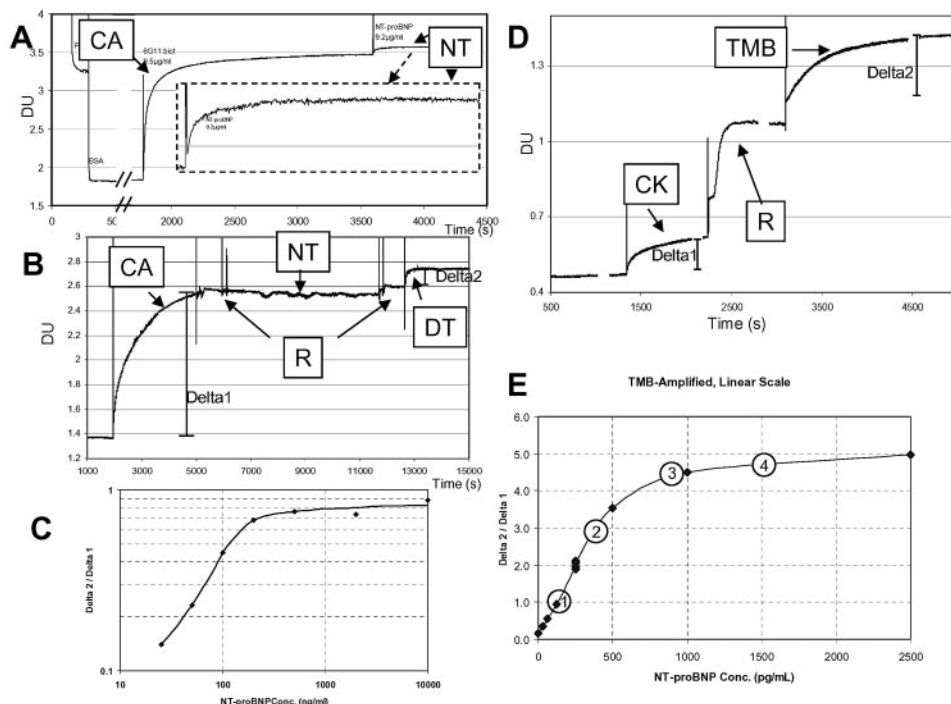


Fig. 1. Detection and quantification of NT-proBNP over a range of concentrations.

(A), real-time trace from the dotLab system, truncated because of space limitations. The dashed box is an enhanced view of the NT-proBNP binding to the immobilized capture antibody. Unprocessed voltage signal from the photodiode reflects the intensity of the diffractive order measured in diffraction units (DU). Arrows indicate introduction of capture antibody (CA) and NT-proBNP calibrator (NT). (B), a real-time trace from the 2 component assays. Bars indicate the approximate location where delta values were derived and used to establish the ratiometric value for each concentration of calibrator. Arrows indicate introduction of CA, NT, detector antibody (DT), and rinse step with PBS or PBS-Tween (R). (C), relationship of delta ratio to NT-proBNP calibrator concentrations. (D), real-time trace from the multicomponent assay. Arrows indicate introduction of mixture (CK) (see Table 1). TMB and R. The large DU increase during the R phase indicates a change in bulk refractive index of the solution from plasma to the PBS washing matrix that did not affect our ability to monitor the subsequent TMB precipitation reaction. Bars indicate the approximate location of the delta values used in the ratiometric determination. (E), relationship of delta ratio to NT-proBNP calibrator concentrations in plasma. Clinical decision points: ①, the clinical cutoff for congestive heart failure (CHF) (1), ② and ③, age-related cut-offs for acute CHF (7), ④, recently suggested cutoff for short-term adverse outcome (8).

membrane peroxidase substrate (Kirkegaard & Perry Laboratories)]. The specific and localized precipitation of TMB on the diffraction pattern causes a signal increase. We again performed internal calibration by use of the ratio of the signal generated by the TMB precipitation to the signal generated by the binding events (Fig. 1D); alternatively, a ratio of binding rates can be used, obviating the need to observe reactions until saturation. We repeated this experiment over a range of calibrator concentrations and generated the calibration curve shown in Fig. 1E. Each point is the mean of a duplicate or triplicate determination. The curve spans 31.25 to 2500 ng/L (~4–300 pmol/L). The lowest value reported is 2 SDs above the mean determination for the zero analyte control. Several clinically relevant decision points are highlighted in Fig. 1E.

In conclusion, we demonstrated that the dotLab system can be used to detect a clinically relevant analyte over a wide range of concentrations in complex media. The observation of real-time binding can reduce the time required to obtain results, because quantification based on rate measurement is inherently more rapid than end-point determinations. For the assay developer, real-time binding observations help identify unwanted reagent interactions that may be sources of background interference or noise. The detection and capture molecules can be modified to quantify other biomolecules such as DNA; we have detected the hybridization of cDNA strands (not shown). Thus, our method lends itself to the detection of many types of analytes.

The simple, robust optics technology at the core of the current compact bench-top platform can be readily deployed, as in the current stand-alone system, or incorporated into various other platforms in the central laboratory and for bedside patient testing.

References

- Hess G, Runkel S, Zdunek D, Hitzler WE. N-terminal pro-brain natriuretic peptide (NT-proBNP) in healthy blood donors and in patients from general practitioners with and without a diagnosis of cardiac disease. *Clin Lab* 2005;51:167–72.
- Morgenthaler NG, Struck J, Alonso C, Bergmann A. Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. *Clin Chem* 2006;52:112–19.
- Tsay YG, Lin CI, Lee J, Gustafson EK, Appelqvist R, Maggini P, et al. Optical biosensor assay (OBA™). *Clin Chem* 1991;37:1502–5.
- Goh JB, Tam PL, Loo RW, Goh MC. A quantitative diffraction-based sandwich immunoassay. *Anal Biochem* 2003;313:262–66.
- Goh MC, Goh JB, McAloney, RA, Loo R. Method and apparatus for assay for multiple analytes. US patent 7 008 794; March 7 2006.
- Goh JB, Loo RW, Goh MC. Label-free monitoring of multiple biomolecular binding interactions in real-time with diffraction-based sensing. *Sens Actuators B Chem* 2005;106:243–48.
- Januzzi JL, Camargo CA, Anwaruddin S, Baggish AL, Chen AA, Krauser DG, et al. The N-Terminal Pro-BNP Investigation of Dyspnea in the Emergency Department (PRIDE) study. *Am J Cardiol* 2005;95:948–59.
- Stanton E, Hansen M, Wijeyesundara HC, Kupchak P, Hall C, Rouleau JL. A direct comparison of the natriuretic peptides and their relationship in chronic heart failure of a presumed non-ischaemic origin. *Eur J Heart Fail* 2005;7:557–65.

A Magnetic Immunochromatographic Strip Test for Detection of Human Papillomavirus 16 E6, Roger B. Peck,^{1*} Johannes Schweizer,² Bernhard H. Weigl,¹ Chamorro Somoza,² Jon Silver,² John W. Sellors,¹ and Peter S. Lu² (¹ PATH, Seattle, Washington 98107; ² Arbor Vita Corp., Sunnyvale, California 94085; * address correspondence to this author at: PATH, 1455 NW Leary Way, Seattle, WA 98107; fax 206-285-6619, e-mail rpeck@path.org)

Cervical cancer kills 230 000 women annually. Low-resource regions of the world are disproportionately burdened with 80% of the cases. Efficient screening methods are the key to decreasing the death toll from this disease. High-risk human papillomavirus (HPV) types have been identified as the etiological agent for >99% of cervical cancers. Infection with HPV is ubiquitous and is often resolved by the host. High-risk HPV infections leading to cervical cancer require the production of both HPV E6 and E7 oncoproteins (1–5). Thus, an assay capable of detecting high-risk HPV E6 from cervical swab samples may have a high positive predictive value and may help to accurately identify women at increased risk of progression to cancer.

Internal studies have shown that cervical cancer cell lines produce ~1 ng of E6 per 1 000 000 cells (data not shown). Work is continuing to quantify E6 amounts in cervical cell samples from clients with cancer, high-grade lesions (cervical intraepithelial neoplasia 2/3), and low-grade lesions (cervical intraepithelial neoplasia 1). Protein determinations performed on provider-collected cervical swabs indicate that the swabs collect 500 000 to 2 000 000 cells.

PDZs (named for the first 3 described domain-containing proteins—postsynaptic density 95, *Drosophila* large disc, and zona occludens) are a conserved class of protein domains that engage in protein-protein interactions by binding PDZ ligands (PLs). PLs usually consist of short C-terminal sequences following the general motif: X-S/T-X-V/L.

Cellular proteins exhibiting PDZ domains fulfill widespread biological functions, including cell-to-cell contact, intercellular signaling, and cell polarity. Numerous viruses encode proteins that have PLs, thus allowing the viral protein to interact with cellular PDZ domain-containing proteins. E6 proteins of only high-risk HPV types interact with PDZs (6–9). Arbor Vita Corporation and PATH, a nonprofit, international health agency, have collaborated on the development of an assay based on the specific interaction of high-risk HPV E6 with PDZ protein and detection via an anti-HPV E6 monoclonal antibody. A sandwich ELISA has been developed and is now being adapted to an immunochromatographic strip platform for use in low-resource settings.

The nearly complete set of PDZ domains present in humans were generated as prokaryotic recombinant fusion proteins and screened for binding to high-risk HPV E6 with an ELISA approach. Fig. 1A shows a subset of the data generated. PDZ 88 demonstrated high affinity to high-risk HPV E6 but not to low-risk HPV E6. E6 PL