

microarray solutions

THE NEXTERION® NEWSLETTER

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In this newsletter:

- ▶ [Low-Density Microarrays with ampliPHOX™ Colorimetric Detection based on Nexterion® Slide AL](#)
- ▶ [Antigen Arrays using APiX Chromogenic Detection System on Nexterion® Slide NC-N](#)
- ▶ [New products - Nexterion® nitrocellulose coated plates in MTP format and incubation chamber](#)
- ▶ [Conference and exhibition calendar](#)
- ▶ [Nexterion® publication list](#)
- ▶ [Nexterion® on Facebook and Twitter](#)
- ▶ [Win a BARNES&NOBLE NOOK](#)

InDevR's Low-Density Microarrays with ampliPHOX™ Colorimetric Detection for Pathogen Identification

Facility: InDevR Inc.
Location: Boulder, CO, USA
Web: www.indevr.com
Substrate used: Nexterion® Slide AL



InDevR, based in Boulder, CO, is a privately held company dedicated to the development of reliable instrumentation and assays for the detection and quantification of viruses and other microorganisms. One of InDevR's innovative products that will be available later this year is ampliPHOX™ Detection Technology, an inexpensive alternative to fluorescence detection for use with low-density microarrays.



The compact ampliPHOX™ Reader provides a colorimetric readout for low-density microarrays in just a few minutes with comparable sensitivity to fluorescence detection

Based on a process called photopolymerization, ampliPHOX is a colorimetric detection method that produces a result in only few minutes. Hybridized microarray targets are first labeled with a proprietary photoactive tag. A solution is pipetted onto the labeled array and exposed to light using the ampliPHOX Reader, a low-cost instrument that performs both the photoactivation and subsequent imaging. Solid polymer spots rapidly form on the array only in locations where target was successfully hybridized to the array. A subsequent staining step helps improve contrast of the polymer and the resulting array can be simply imaged. In addition, an intuitive software package automates the quantification and results interpretation.

InDevR is also developing several low-density microarrays for pathogen detection to couple with the ampliPHOX platform. In a recent review article [1], Mikhailovich and colleagues argue that the most promising clinical application of microarray technology is in the surveillance and diagnosis of infectious diseases. With recent advancements in sequencing large databases of full genome sequences exist for thousands of viral and bacterial pathogens and can be used to design extensive microarrays [2]. There is no doubt that clinical science has clearly been advanced by the use of high-density gene expression microarray studies in a variety of fields. However, the overall complexity, high cost, and regulatory challenges associated with high-density array technologies may be contributing factors as to why the adoption of microarrays in the clinical environment has been slow [2]. New methodologies that can simplify the technology and data analysis as well as reduce overall assay cost will provide an avenue for introducing the power of microarrays for infectious disease diagnosis into the clinic.

An example of one diagnostic microarray currently under development at InDevR is the FluChip. Originally developed at the University of Colorado, InDevR licensed the technology in April 2009. Although clinical use is the ultimate end goal, InDevR plans to commercialize the FluChip later this year for influenza surveillance and other research uses. Unlike higher density two-color gene expression arrays, the FluChip assay requires only a yes/no answer for the presence of each target on the array, greatly simplifying the quantification of the final result.

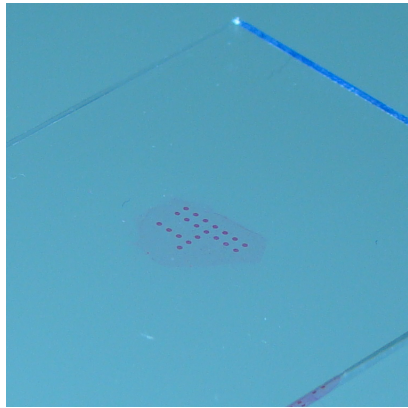
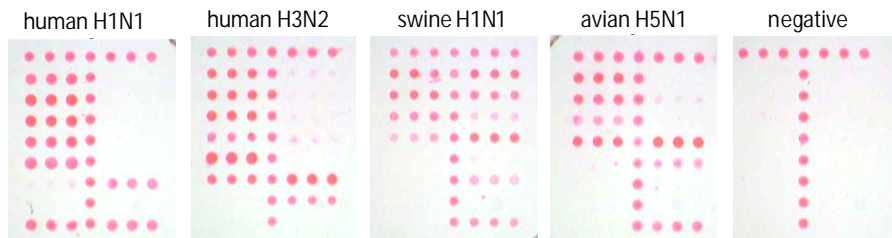


Image of an ampliPHOX detection result on an early version FluChip printed on a SCHOTT Slide AL.

The FluChip consists of a small number of amino-terminated capture probes (<20 total) and a positive control sequence are each printed in triplicate. The substrate we have chosen for this work is the SCHOTT Nexterion® Slide AL. Using slides from other manufacturers, we battled lot-to-lot inconsistencies and difficulties with spot morphologies. These inconsistencies made it very difficult to optimize the overall process, as we could not rely on two successive batches of chips to produce a similar result. After speaking with another trusted company and learning of their positive and professional interactions with SCHOTT, we decided to test out the Nexterion® Slide AL product. We have been happy with the switch, as we have found the SCHOTT substrates to be very reliable, producing consistent results lot-to-lot.

The FluChip assay begins with nucleic acid extraction from a clinical specimen, and subsequent amplification via a multiplexed RT-PCR assay to amplify the influenza and internal control targets. This step also serves to incorporate biotin into the target and prepare the target for a subsequent enzymatic digestion step. A quick enzymatic digestion and fragmentation step is performed, and the target is hybridized to the microarray for 30 minutes at room temperature. After a series of washing steps, the ampliPHOX detection protocol is performed as described above, with the detection process taking about 10 min and the entire assay from start to finish taking only ~5 hours. With ampliPHOX detection, the end result is an easy visual readout where the overall detection patterns are indicative of the influenza A subtype present, as shown in the accompanying figure. An intuitive software package is used to quantify the signals relative to background, automate interpretation of the images, and provide a result to the user.



Specimens were processed using a prototype FluChip protocol and ampliPHOX detection. Influenza A subtypes are shown above each image. Images collected with the ampliPHOX Reader.

For more information about ampliPHOX™ Detection Technology, the FluChip™, and InDevR's other exciting available technologies, visit: www.indevr.com

References

1. Mikhailovich, V., Gryadunov, D., Kolchinsky, A., Makarov, A. A., Zasedatelev, A. (2008) "DNA microarrays in the clinic: infectious diseases", *BioEssays*, 30: 673-682.
2. See National Center for Biotechnology Information (NCBI) website at :
<http://www.ncbi.nlm.nih.gov/sites/genome>
3. Kumar, A., Opel, M., Moore, M., Baunoch, D. (2006) "DNA microarrays in IVD applications", *IVD Technology Magazine*, Nov. 1 issue.

Cancer / Testis Antigen Arrays using APiX Chromogenic Detection System on SCHOTT Nexterion® Slide NC-N

Facility: InDevR Inc.
Location: Boulder, CO, USA
Web: www.indevr.com
Substrate used: Nexterion® Slide AL



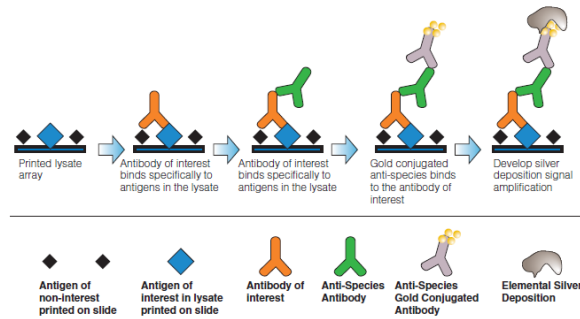
Overview

Cancer / testis (CT) antigens are immunogenic proteins normally expressed only in germ cells such as the testis. Recent discoveries have shown that CT antigens are also expressed in tumors from a variety of cancers and that CT antigens are promising vaccine targets. Immunoglobulin G (IgG) antibodies generated in response to CT antigens can be sensitive and specific serum biomarkers for the early detection of disease, patient selection, the prediction and monitoring of drug reactions, drug repurposing and applications in translational medicine.

Gentel's CT Antigen array on the SCHOTT Nexterion® Slide NC-N slide is a new protein array-based tool that enables the simultaneous measurement of changes in serum antibody content against up to eighty CT antigens. These arrays can be probed with serum, for detection, scanning, and analysis using the Gentel APiX View Chromogenic Detection system, GPM scanner and AthenaQuant Software.

Protein arrays enable immobilization of proteins in their native conformations and samples can be run under aqueous conditions instead of reducing conditions. Gentel's CT Antigen array is compatible with standard well plate configurations and can use automated protocols developed for the ELISA format.

Gentel APiX View Chromogenic Detection system and GPM offers further benefits with simple off the shelf protocols and applications that are highly sensitive, robust and affordable. APiX uses a proprietary gold-catalyzed silver deposition and typically achieves measurably improved sensitivity compared to fluorescence detection. SCHOTT NC-N slides were selected for their high binding capacity, customizable pad formats, low background and compatibility with the GR8 scanning system.



Schematic showing the application of APiX Detection system used in conjunction with CT antigen protein arrays on the SCHOTT NC-N slide

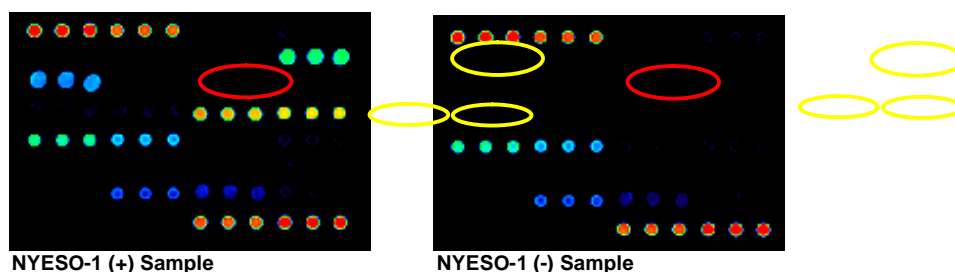
Methods

Protein arrays are manufactured by printing CT antigen HEK293T over-expressed cell lysates in a 2 x 8 sub-array format onto SCHOTT Nexterion® Slide NC-N16 slides using non-contact piezo-electric arraying and then blocked. In this particular example, each sub-array contained 10 unique CT antigen proteins printed in triplicate; MAGEA1, SSX4, MAGEB6, CTAG1B, MAGEA4, SSX2, NLRP4, MAGEB1, GAGE7, NXF2 and NYESO-1. Current CT array offerings can include array densities of up to 80 CT antigen lysates in triplicate.

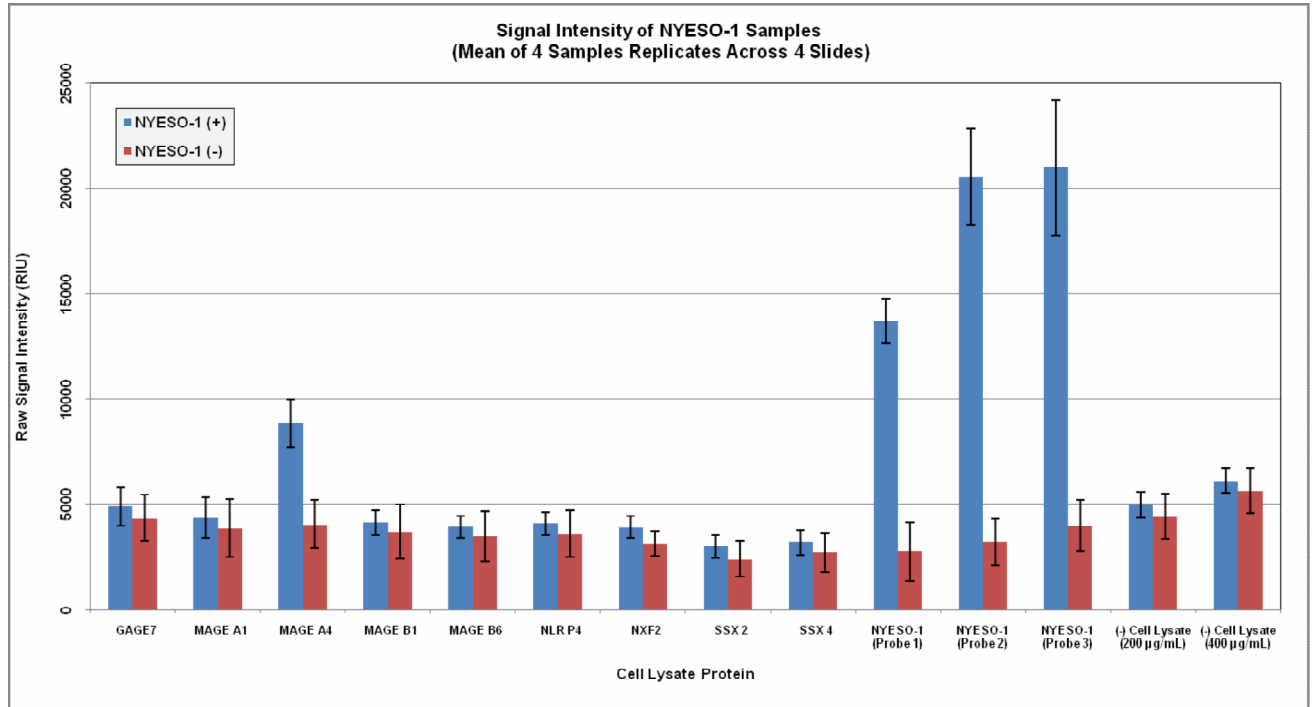
Two samples were obtained from a clinical collaborator consisting of known normal and validated NYESO-1 positive human serum. Processed slides were scanned on a Gentel Proteomics System in Reflection Mode (White Surface) and saved as a .tif file. Array spot and local background signal values were extracted using Gentel AthenaQuant V.1.1.

Results

Both NYESO-1 positive and NYESO-1 negative samples showed excellent signal-to-noise and clear differentiation. The immune response to NYESO-1 as measured by the array is very strong and consistent for the sample previously characterized as NYESO-1 positive for all three NYESO-1 probes. An immune response to MAGE A4 is also observed in the NYESO-1 positive sample. While the response to MAGE A4 has not been validated by ELISA, this response is not unusual for this sample type.



False color images of 2 sub-arrays on the processed and developed microarray slide. NYESO-1 positive sample is on the left, NYESO-1 negative sample is on the right. Array spots showing NYESO-1 lysate spots highlighted by the yellow ovals, MAGEA4 show with the red ovals. The remaining spots on the array showing signal response are positive controls.



Graph showing the raw signal intensity response of the NYESO-1 (-) and NYESO-1 (+) samples to the CTA proteins on the array along with error bars (n=4)

Reproducibility was evaluated by measuring replicate NYESO-1 (+) samples across four replicate sample wells (intra-slide CV) and 4 separate slides (inter-slide CV). Consistency within slides was typically less than 15% and mean inter-slide %CV measured 14.7%.

Mean Signal Variation for NYESO-1 (+) Sample		
Protein	Mean Intra-Slide %CV (N=4 replicates, 4 slides)	Mean Inter-Slide %CV (N=4 slides)
GAGE7	12.3	18.3
MAGE A1	14.1	22.2
MAGE A4	12.0	13.1
MAGE B1	13.1	13.9
MAGE B6	15.7	13.5
NLR P4	14.9	13.1
NXF2	14.0	14.2
SSX 2	16.1	17.8
SSX 4	17.8	18.0
NYESO-1 (Collaborator)	9.8	7.7
NYESO-1 (200 µg/mL)	8.6	11.0
NYESO-1 (400 µg/mL)	12.2	15.4
(-) Cell Lysate (200 µg/mL)	9.7	12.4
(-) Cell Lysate (400 µg/mL)	10.9	9.7
Mean	13.1	14.7

Table showing summary of precision experiments for NYESO-1 (+) samples

Conclusions

There is increasing evidence that profiling tumor-associated antibodies can be an effective strategy for biomarker discovery, profiling, and identification of new vaccine targets. Cancer-testis antigens are of particular interest as potential target proteins given that their expression is typically restricted to germ cells among normal tissues, but aberrantly expressed in multiple tumor types. This successful application demonstrates the use of a CT antigen protein array, SCHOTT Nexterion[®] nitrocellulose slides and APiX View detection system yields excellent results both in terms of sensitivity, reproducibility, and correlation with previously obtained results. Coupled to the very low cost of the platform, Gentel offers a powerful tool for the biomarker researcher seeking to increase throughput, reduce costs, and accelerate their discovery.

New products - Nexterion[®] NC MTP microarray plates and incubation chamber

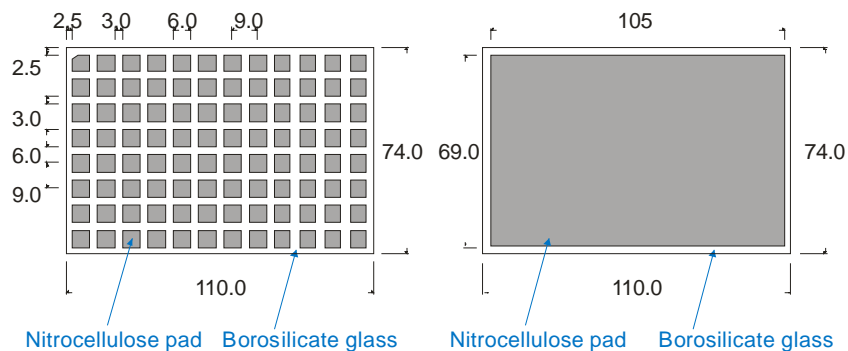


Nexterion[®] MTP-NC96 is an innovative 96-well microplate platform for parallel analysis of multiplexed assays. The product combines all the advantages of the nitrocellulose coating with the high-throughput 96-well microplate format; making automated parallel assays a reality. MTP-NC96 conforms to the SBS standard microplate format commonly used in clinical diagnostics, high throughput screening and drug discovery. SCHOTT's innovative two-component system allows users to array all types of probes on a flat, planar surface, and then create a 96-well microplate after arraying. The coating is manufactured using an advanced nitrocellulose casting method, for a highly reproducible surface

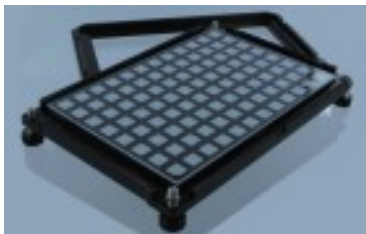
with low intrinsic background fluorescence. The Nexterion[®] MTP-NC96 is available either as a large single pad format plate, or a 96-well format specifically designed for high-throughput use.

The two main components comprising the MTP-NC96 system are available as separate items.

1. High quality nitrocellulose coated glass plates



2. Nexterion® IC-96 Incubation chamber and plate holder



The reusable 96-well incubation chamber consists of a flexible, black silicon superstructure held within a high quality, black anodized aluminium holder. The silicone superstructure is tightly held against the glass slide surface by two aluminium plates to create a leak-proof seal around each well. The press-fit silicone superstructure eliminates the need for adhesives that are autofluorescent, and can increase background signal of the slide coating. The chamber is designed to be re-usable, replaceable superstructure is available to maximise the

usable life. Note: The Nexterion® IC-96 will only fit glass plates that have the dimensions 110.0 x 74.0 mm. With care, it is possible to remove the dividing well walls of the superstructure, so that the IC-96 may also be used with the single pad of the MTP-NC-C/N plates.

Conference and exhibition calendar

Come and meet the Nexterion® Team at the following conference

Conference	Location	Dates
Microarray World Congress	San Diego, CA, USA	28 - 29 October 2010
Functional Genomics - Next Generation Applications and Technologies	Frankfurt a. M., Germany	03 - 04 February 2011

Nexterion® citation list

We are regularly updating our list of scientific literature that cite the use of Nexterion® microarray products. The publications are categorized by the type of slide coating used, probes printed and application. It is a useful guide when deciding which of the Nexterion® coatings to use with any particular application.

Up to now the number of scientific publications increased to more than 600. The list is available from the SCHOTT web page at:

http://www.us.schott.com/nexterion/english/download/nexterion_publicationlist.xls

Nexterion® on Facebook and Twitter

Maybe you already noticed it: we are now part of the Twitter and Facebook communities. We are using these media to better interact with you and want to inform you about:

- new products and developments at SCHOTT
- new publications using Nexterion® products
- posting news from across the industry that is relevant to you
- interesting details about our daily work

To follow us, simply go to Facebook or Twitter and search for Nexterion.
We are looking forward to welcome you in the Nexterion® community.

Nexterion® Contest

Win a BARNES&NOBLE NOOK e-book reader.

By entering our competition, you will have the chance to win a BARNES&NOBLE Nook e-book reader. Simply answer the question below, and send your answer to arrive before 12 midnight (CET) on 31 December 2010.

By email: coatedsubstrate@us.schott.com
By fax: + 49 (0) 3641 681 970

The winner will be the first correct entry drawn at random.

(Only one entry per person. The judges' decision is final. This competition is void where prohibited. This competition is run and staged in accordance with German legislation.)

Question:

What is the thickness of the nitrocellulose membranes on SCHOTT Nexterion® NC slides and plates:

- a) 8 microns
- b) 11 microns
- c) 15 microns

(Find the answer at our website www.schott.com/nexterion)

Impressum

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