Published in final edited form as:
Anal Chem. 2010 March 1; 82(5): 1571–1579. doi:10.1021/ac901743u.

Programmable Nano-Bio-Chip Sensors: Analytical Meets Clinical

Jesse V. Jokerst
The University of Texas at Austin

Pierre N. Floriano, **Nicolaos Christodoulides**, and **John T. McDevitt**
Rice University

James W. Jacobson and **Bryon D. Bhagwandin**
LabNow, Inc.

synopsis

There have been many recent advances in the nano-bio-chip (NBC) analysis methodology with implications for a number of high-morbidity diseases including HIV, cancer, and heart disease. In their Feature article, Jesse V. Jokerst of The University of Texas at Austin; Pierre N. Floriano, Nicolaos Christodoulides, and John T. McDevitt of Rice University; and James W. Jacobson and Bryon D. Bhagwandin of LabNow, Inc. discuss the construction, capabilities, and advantages of NBCs. The cover shows arrays of NBCs. Images courtesy of Glennon Simmons/McDevitt Lab and Marcha Miller of The University of Texas at Austin.

Clinical analysis remains one of the most important frontiers in measurement science as an ever-increasing understanding of living systems places evolving demands on the bioanalysis laboratory.[1, 2] One important trend is toward miniaturized designs—for clinical systems, this can lead to medical results at the point-of-care (POC), i.e., bedside, ambulance, or other remote location.[3] Despite remarkable progress toward POC clinical assay systems, very few complete working prototypes have emerged. Although promising starts have been made with

Jesse V. Jokerst recently completed his Ph.D. in chemistry with Professor McDevitt at The University of Texas at Austin, where he created improved NBC signaling approaches. He is currently developing multimodal nanoparticles for in vivo measurements as a NIH R25T postdoctoral fellow in the labs of Sanjiv Sam Gambhir and Shan X. Wang within Stanford University's Molecular Imaging Program. Pierre N. Floriano is a senior scientist at Rice University, where he directs McDevitt lab microfluidic, imaging, and data analysis. His research interests are the design and fabrication of integrated cellular and proteomic microchip approaches and custom image analysis algorithms for image-based sensors. Nicolaos Christodoulides is a senior scientist in the McDevitt group at Rice University, where he directs immunoassay development. His interests are aligned with his background in cardiovascular disease and immunology, and his research develops medical devices based on the bead- and membrane-based NBC assay platforms. James W. Jacobson is currently Vice President and Chief Scientific Officer at LabNow, Inc. and has over 20 years of experience in research, development and commercialization of research and medical products. He also directed high-complexity CLIA certified laboratories for more than four years. Dr. Jacobson has a Ph.D. in Population Biology (Genetics) from Washington University in Saint Louis and has authored publications in genetics, molecular biology, and laboratory science. Bryon D. Bhagwandin has spent more than 20 years designing and developing medical instrumentation. He has two degrees in physics and earned his doctorate in bioengineering from the University of Washington. His experience and expertise include design of portable and handheld medical-instrument systems, biosensors, image analysis, biomedical optics, software system-control design, microfluidics, and small company management. Currently, he is the president of LabNow. John T. McDevitt earned his doctorate in chemistry from Stanford University. After moving from The University of Texas at Austin, he recently joined the Rice faculty as the Brown-Wiess Professor of Bioengineering and Chemistry. He and his research group strive to develop micro-medical devices that are affordable and accessible. McDevitt serves as scientific founder for LabNow, which targets the release of HIV immune tests in resource scarce settings. Contact McDevitt at Department of Chemistry Rice University 6100 Main Street, Houston, TX 77005 (mcdevitt@rice.edu).

COMPETING INTERESTS

John T. McDevitt serves as the scientific founder for LabNow, Inc.. The authors have applied for patents in areas related to NBC sensor systems.

There have been many recent advances in the nano-bio-chip analysis methodology with implications for a number of high-morbidity diseases including HIV, cancer, and heart disease. (To listen to a podcast about this article, please go to the *Analytical Chemistry* multimedia page at pubs.acs.org/page/ancham/audio/index.html.)

microfluidic lab-on-a-chip (LOC) approaches and important goals defined with the micro total analysis system (μTAS) paradigm, the broad-scale release of workable devices has yet to be achieved.[4, 5] While their analysis core is substantially smaller than benchtop alternatives, the network of macroscopic laboratory-based infrastructure required for sample processing, analyte detection, data processing, and reagent handling implies that these platforms are best described as "chips-in-a-lab" rather than true "labs-on-a-chip".[6] The absence of a standard and modular analysis technology that spans multiple analyte classes motivates work toward universal mini-detection ensembles amenable to rapid prototyping with easy inclusion of newly validated biomarkers.

The POC analysis solution described here, the programmable nano-bio-chip (NBC), synergizes components and achievements from nanotechnology, clinical chemistry, bioinformatics, microfluidics, optics, image analysis, and pattern recognition to create a powerful new integrated measurement approach in a small device footprint. The NBC ensemble employs a size-tunable network of nanometer-scale fibers (a "nano-net") within agarose microspheres or a polymer membrane and a fluorescent signal arising from nanoparticles (nano) to isolate and quantify biologically important analytes (bio) from complex matrices within a closed, miniaturized system (chip).[7-9] The NBC features a flexible assay design and has a diverse collection of validated analyte subtypes. The NBC's modular design elements allow for rapid inclusion of tests for new biomarker signatures; assays for nucleic acids, proteins, and cells are arranged in the NBC to create analytical test modalities specific to different disease types. Collectively, the modularity, flexibility, and ability to process and learn new biomarker signatures is here referred to as "programmability". This Feature article describes the creation of the "programmable NBC system" as a universal, modular, clinical analysis system, typical results, and the opportunities presented by this integrated approach.

BACKGROUND AND GOALS OF CLINICAL ANALYSIS

Clinical samples are diverse and dynamic; in the past, this has precluded the facile development of universal analysis systems. Though a state-of-the-art clinical lab can measure a few thousand unique species, a typical blood sample contains hundreds of thousands of different analytes when nucleic acid rearrangements are considered.[10] Additionally, the variety of material contained in blood—cells, proteins, nucleic acids, small molecules, and ions, which each require a different isolation and detection method—further complicates clinical testing.[11, 12] Finally, high-quality reference materials are required for standardization, yet very few clinical assays have NIST-certified control substances.

A number of experimental approaches have been developed to satisfy these clinical needs. Though the scope of this article precludes comprehensive discussion, the contributions of a few key groups deserve mention (see more complete reviews for further analysis[5, 13, 14]). Whitesides's fundamental studies of self-assembled monolayers helped define the ideal materials, coatings, and designs required to create microchannels and manipulate biological fluids.[15] Later work integrated advances in nanotechnology into existing systems, and recent papers described microfluidic structures constructed only of paper and tape.[16] Similarly, Quake's research has advanced the "large scale integration" of microfluidics;[17] this key experimental platform has been used to explore genetic and protein applications, as well as the biophysical properties of single molecules. Ligler has worked extensively in biodefense applications, while others, such as Mirkin, Lieber, and Wang, use precious metal nanoparticles and magnetic techniques to measure diverse sample types and create a variety of assembly types.[18-21]

Other approaches by Sia with MEMS and Singh using chip-based separation and quantitation have continued to increase integration.[22, 23] Though the i-STAT platform was the first POC

sensor array to be microfabricated and sold commercially as a whole-blood diagnostic tool, the system is limited to a few non-cellular analytes.[24] Other assembled systems, though nearing completion, have not yet been released for widespread clinical use. Work by Madou and others has resulted in the LabCD, which eliminates traditional valves and pumps by using centrifugal and centripetal force to perform fluid movement.[25] Walt's work with electronic noses uses arrays of optical fibers as the underlying infrastructure for biological sensing systems.[2]

CONSTRUCTION OF THE NBC

Over the past decade, the NBC approach has become increasingly sophisticated in making a variety of biomedical measurements.[7] Designed originally as an "electronic tastechip", the NBC contains biologically inspired recognition elements, as well as pattern recognition features, to identify multiple analyte classes in complex fluid samples similar to gustatory excitation processes that occur at mammalian taste buds.[7]

Different foodstuffs rarely contain a single dominant tastant (analyte resulting in taste perception) but rather are composed of a combination of sweet, sour, salt, bitter, and umami elements, which stimulate various types of taste buds. Foods and beverages are recognized by the gustatory fingerprint response. Building on this key inspiration—complex fluid analysis with pattern recognition and refined decision making after experience learning has occurred —the programmable NBC is a flexible platform that adapts quickly to new inputs to refine the decision-making capabilities related to human health. Here, analyses of many different cell types, proteins, and small molecules in combination are often required to identify the bio-fingerprints corresponding to disease states. Importantly, this systems level approach can learn; the interpretation of individual assay results is trained in the NBC. This digital self-modification via sensors specific to a variety of analytes may eventually be a powerful replacement for the disease-specific design of many current POC approaches.

The modular NBC employs two central reaction unit types, each with tailored applications. The first uses a micro-membrane filter integrated into a fluidics structure (Figure 1ai-ci). This membrane can be considered a "cellular processing unit" because it performs cell counting/characterization similar to flow cytometry (FC), albeit with a markedly dissimilar mechanism.[26] A second class of reactor uses a microbead array wherein indentations within a silicon or stainless steel wafer are populated with chemically sensitized bead reactors. This "chemical processing unit" (Figure 1aii-cii) selectively isolates and quantitates macromolecules from complex sample types.[9] Both NBC sensor ensembles have been adapted for a broad range of analyte classes.[18-21]

Four aspects of the NBC should be noted: quality analytical behavior, a programmable (modular) design, the breadth of function, and the inexpensive nature of the analysis ensemble. First and most importantly, NBC methods meet or exceed the analytical characteristics (sensitivity, selectivity, assay variance, and limit of detection [LOD]) of mature instrumentation for a wide variety of analyte systems. For example, Table 1 details the operational features of the inflammation marker C-reactive protein (CRP) NBC assay versus other gold-standard systems.18,22 The NBC's capacity to rapidly (10 minutes) detect analyte across several orders of magnitude with a LOD of 10 fg/mL (88 aM) is attributed to the unique analyte preconcentration feature of the agarose bead, high signal, and low background due to efficient washing, a function of microchip design and the bead structures that have tailored macro- and micro-pores reminiscent of a "microsponge". Second, the modular design of the NBC allows easy creation of custom assay panels by matching bead types to the end application without requiring changes to the microfluidic elements or bead support structure. Validated bead types are loaded when needed in any desired combination. This "plug and play" feature uses both the spatial code of bead placement and the molecular-level code present in reagents.

Third, the simplicity with which the NBC transitions from cellular experiments to a miniaturized protein immunoassay platform is unique; prior efforts also have combined these two approaches serially.27–29 Finally, the use of microfabrication, parallel processing, and inexpensive construction materials could make analysis highly cost effective. This "budget-conscious design" was recently demonstrated for use in resource-poor settings and has relevance to the renewed national dialogue over healthcare affordability.26 The coherent behavior of elements from the nano-, bio-, and chip-based arenas of research contribute to the high quality analytical performance of these NBC systems. The unique roles these three elements play in the analysis scheme are detailed below.

NANO ADVANTAGES: BIG ATTRIBUTES OF SMALL-SCALE CONTROL

For the multiplexed signaling requirements of the NBC, the long Stokes shift and high intensity of quantum dots (QDs) combined with their narrow emission profiles and simultaneous excitation makes these inorganic fluorophores especially attractive. When compared to AlexaFluor 488, QDs yield 30× signal amplification.[30] This increase in intensity in tandem with NBC design yielded LODs two orders of magnitude lower for carcinoembryonic antigen (CEA) (21 pg/mL) than the conventional enzyme-linked immunosorbent assay (ELISA) and one order lower than NBC-AlexaFluor.30

Additional nano-tuned elements within the NBC include the net of agarose pores within the bead microsphere. A customized synthesis process created a catalog of beads with tunable hydrophilicity, diameter, and reactive groups.[31] Those most commonly used are 2% (wt./wt.) agarose with pore diameters of 120–160 nm—ideal for most analyte classes because they most efficiently preconcentrate analyte from the sample. Beads with larger pore sizes do not retain structural rigidity and deform in the microfluidic flow chamber, whereas beads with smaller pores lose their "sponge-like" character and begin to behave as a solid spherical surface. Finally, the size of recognition elements has been tailored to include both ~15 nm IgG monoclonal antibodies and smaller (3 nm) single chain variable fragment or aptamer capture and detection bodies. Initial work in this area indicates that beads are functionalized with an even higher density of these immobilization tools than whole IgGs.[32]

BIO ADVANTAGES: PERSONALIZED ANALYTES

Because the NBC is quickly programmed with beads specific to a broad range of analytes, the approach has the potential to keep pace with the ever-increasing knowledge base in molecular diagnostics. Currently, research groups and even entire institutes work to mine serum for new protein biomarkers and uncover their relevance in disease.[33] Alternative studies have begun to unravel the myriad proteins present in saliva.[34] Thousands of such biomarkers are known, and many more are constantly discovered through chromatographic/mass spectrometric methods with the hope of using them for screening and treatment evaluation.[33] More recently, with characterization of the human genome, the complexity of information needed to arrive at an accurate diagnosis increased further. The 500,000 to 1 million single nucleotide polymorphisms available in genome-wide association studies make processing incredibly large amounts of information critical.[10] No longer are single assays the dominant theme for diagnosis; rather, the requirements for disease identification is shifting now to a battery of analyses. Moving beyond biomarker discovery into the realm of clinical practice, however, is a key challenge for next generation diagnostic devices. The NBC satisfies this need by using the same miniaturized fluid-handling approach, but with beads specific to the desired analytes programmed into this core. Thus, the NBC could help move the biomarkers from discovery through validation and on to clinical implementation, which establishes the true benefits to society. Using the same fluid and light handling equipment across all of these assays minimizes development times and costs and maintains universality.

CHIP ADVANTAGES: LESSONS LEARNED FROM MICROFLUIDICS

The NBC uses the microfluidic chip advantages of miniaturized sample and reagent sizes and inexpensive construction materials to complement its nano and bio attributes. By definition, microfluidic structures manipulate small amounts of material through various analysis and sensing steps.[8, 33-36] Reduced sample and reagent volume requirements and smaller amounts of construction materials decrease costs drastically, and assay times may also be cut substantially. Microfluidic devices' ability to isolate and concentrate analyte, as well as their capacity for mixing, allotment, and fluid focusing, decreases the number of sample-handling steps. These architectures can perform separations with detection elements designed in-stream on a small size scale, which makes them highly compatible with next-generation analysis platforms.[1, 13]

Still, microfluidic systems have not been the panacea for analytical challenges envisioned a decade ago.[6] First, most applications are not fully freestanding and require extensive support networks of compressed gases and liquids, electronics, syringes, external pumps, and any necessary optics and light handling hardware. Second, PDMS, a common skeleton of microfluidic devices, has not been translated into rapidly-prototyped systems because of hydrophobicity and nonspecific absorption of bio-molecules other than the analyte.[35] Third, because devices may become fouled, microfluidic structures may need be disposable, which limits the cost-effectiveness of some approaches.[36] Finally, challenges with overall scalability —whether lowering the volume or increasing the throughput—have prevented more complete development. Microfluidics is an important *tool* for analytical device construction but is not the be-all-and-end-all replacement for traditional systems that was originally envisioned.

INTEGRATION: ASSEMBLY OF WORKING PARTS

Although individually valuable, new biomarkers, advances in microfluidic condensation, and novel nanoparticle bioconjugates are most elegant within rational, integrated designs.[39-41] As Ligler recently observed, ideal system designs are best described as "Gestalt": the configuration of the whole possesses attributes unattainable by the summation of the individual parts.[37] Moving the chip out of the lab will require new analytical concepts that bridge many different size scales and employ the nano-, bio-, and chip-based attributes (Figure 1).

This ensemble consists of a closed sample loop that immobilizes specific analytes from complex matrices using an antigen-specific bead array or a size-specific membrane and quantitates them via a colorimetric or fluorescent signal. This physical design has been documented previously.12, [17] The two processing units, cellular (membrane) and chemical (bead), are illustrated in Figures 1c(i) and 1c(ii), respectively; Figures 1a(i) and 1a(ii) display a molecular-level view of the reactor cores. Importantly, both types integrate easily into the compact NBC labcard (1d) and can be interpreted by the standalone analyzer (1e).

The integrated design contains all elements required for a complete assay, condensed into a credit-card-sized disposable cartridge. Figure 2 contains a photograph of the analyzer that interfaces with these disposable cards; it has a footprint about the size of a toaster and costs about one-fifth of the current macroscopic instruments. Blister packs hold the liquid reagents, and the solid form of fluorescently-tagged biorecognition moieties are on the back of the labcard. Channels designed for mixing and fluid flow permeate this architecture, and manipulations of the fluidic cartridges reconstitute and disperse reagents through the labcard. Linear actuation controls all fluid motion via pressure actuation steps.

The cards are constructed from common, inexpensive materials, including vinyl adhesive, laminate, stainless steel, and poly(methyl methacrylate) (PMMA). Computer-aided design (CAD) models the cards, and then a CAD plotter/cutter incises the vinyl. Up to seven layers

of vinyl/laminate are deposited on six to eight cards using conventional, parallel layering methods. Cards are disposable and purposed to service one patient.

Either venipuncture or finger-stick for blood or simple expectoration for saliva are used to collect samples. For finger-sticks, the whole blood sample briefly resides in a capillary tube before introduction into the NBC via capillary action. The labcard is then inserted into the analyzer, where perturbations of the fluid-containing blister packs complete the assay. Optical signal capture occurs via a magnifying objective, and assay output displays on a built-in screen shortly after completion. The device contains AC/DC power supplies with a battery life of several hours, and downstream processing software, readout display, memory for up to 50,000 patient histories, and USB/Ethernet/wireless communication features complete the device. Total weight is 13.5 lbs, making the system amenable to measurements at the POC. The following sections detail the results obtained using this analysis ensemble or a similar research-grade prototype.

PARTICULATE PROCESSING

One of the initial applications for the NBC system is the analysis of T lymphocytes in HIV positive patients—crucial because the enumeration of CD3+CD4+ cells is required to determine immune system status and efficacy of antiretroviral treatment.[26] Using this simple cell capture mechanism, the polycarbonate, track-etched membrane retains the larger and more rigid lymphocytes while allowing blood matrix components including plasma, platelets, and erythrocytes to pass to a waste reservoir. Figure 3 illustrates the coding of CD3 surface markers in red (a) and CD4 in green (b), resulting in dually-stained cells (c); these are quantitated using custom automated image analysis algorithms. Simultaneous visualization of differently-colored nanoparticles via QDs (Figure 3d) reduces the optical requirements significantly because molecular fluorophores require filters specific to each color channel.[38] In pilot studies (n = 200), the NBC correlated nicely ($R^2 = 0.94$) to FC, the gold standard method, while maintaining baseline separation between CD4+ lymphocytes and monocytes. This level of performance suggests that the device provides reliable results from finger-stick sized samples (30 L) in <20 minutes (Figure 3e-f). This technique is also amenable to pediatric HIV immune function testing (% CD4), total white blood cell counts, and differential white blood cell testing.[27]

Independent of analyte identity, converting electronic photomicrographs into cell counts uses digital image processing and customized deconvolution schemes. To optimize the software for correct photomicrograph interpretation, fluorescent beads with physical dimensions similar to lymphocytes were used as were authentic lymphocyte samples. Through an iterative process, such descriptors as size, circularity, and signal-to-background ratio were trained on nearly 1 million individual cellular events, which resulted in cell counts statistically identical to FC. The difference between each field-of-view is <10% and indicates that the arrangement of cells on the membrane is more or less random and free of bias in the absence of gross error. The variance between repeated analyses of the same sample is 5–8%. This approach has been validated to a LOD of 50 cells/μL and up to 600 cells/μL, which includes both the region where increased testing is recommended (200–600 cells/μL) and the region considered active AIDS (<200 cells/μL). Cellular crowding on the membrane leads to inaccurate counting and hampers cell counts >600 cells/μL; overlap leads to exclusion from digital analysis. Photomicrographs containing >20% of active pixels generally had a large number of overlapping lymphocytes.

Though overlapping events are easily translated by advanced algorithms, an alternative method also circumvents this shortcoming at high counts. Here, total membrane fluorescence extended the linear dynamic range in lymphocytes and spore counting applications by three orders of magnitude.[26, 29] This system achieved detection limits of ~500 *Bacillus globigii* (a commonly

used simulant for *Bacillus anthracis*) spores, a value highly competitive with similar technology, but on a shorter timescale (~5 minutes).[29]

Additionally, a new automated cellular analysis method for examination of molecular and morphological biomarkers associated with oral carcinogenesis contributes to the early detection of oral cancer. Four cellular features were indicators of oncogenesis: nuclear area and diameter, nucleus-to-cytoplasm ratio, and epithelial growth factor receptor (EGFR) biomarker expression. Examination using linear regression and receiver operating characteristic (ROC) curve analysis identified the morphological features as the best predictors of disease, though a combination of all features is ideal for classification. These EGFR assays yielded results in <10 minutes with staining intensity, homogeneity, and cellular localization patterns comparable to conventional methods. Further study of EGFR expression in three oral cancer cell lines indicated a significant increase ($p < 0.05$) above control cells. Results obtained in the NBC correlated nicely with FC ($R^2 = 0.98$) and detected important differences ($p < 0.01$) between two of the oral cancer cell lines: a disparity of ~34,000 EGFR reporters per cell according to quantitative FC.39 Cells with ≥2500 copies of a given receptor could be detected with the NBC. Using this same NBC system, a pilot study 40 was completed recently to explore the clinical efficacy of the approach. Here, oral lesions from 41 dental patients, along with normal epithelium from 11 healthy volunteers, were sampled using a non-invasive brush biopsy technique. Four key parameters were significantly elevated in both dysplastic and malignant lesions relative to healthy oral epithelium, including the nuclear area and diameter ($p < 0.0001$), the nuclear-to-cytoplasmic ratio ($p < 0.0001$), and EGFR biomarker expression ($p < 0.03$). Further examination using logistic regression and ROC curve analysis identified morphologic features as the best predictors of disease (area under curve [AUC] ≤ 0.93) individually, while a combination of all features further enhanced discrimination of oral cancer and pre-cancerous conditions (AUC = 0.94) with high sensitivity and specificity.40

Taken together, these results indicate that the NBC cellular processing unit is a versatile platform and appropriate for rapid detection of HIV-relevant lymphocytes, bioterrorism threats, and oral cancer biomarkers.

MACROMOLECULAR MEASUREMENT

The use of oral samples for disease monitoring extends beyond cellular analytes. Although serum has a long history as a diagnostic medium, the recognition of oral secretions for clinical analysis is relatively nascent. Increasing evidence suggests that saliva is a “mirror on the body” and contains signaling molecules related to cellular and systemic disease.[41] The facile gathering and storage of saliva appeals to clinicians, and the noninvasive collection method reduces anxiety and discomfort for patients. In addition, as a constantly regenerated fluid, saliva in some circumstances may offer a better “physiological snapshot” of human health.[42]

Two of the most extensively explored clinical areas by the chemical processing NBC are heart disease and cancer, the current number one and number two causes of death in developed countries, respectively. The progression of cells from healthy to cancerous alters the genomic and proteomic makeup of tissue; early detection of these changes remains key to decreasing morbidity.[43] Studies of CEA, Her-2/*Neu* (c-erbB-2), and cancer antigen 125 (CA125) (with correlation to colon, breast, and ovarian cancers, respectively) have turnaround times on the NBC of <30 minutes, four times shorter than a typical ELISA. An extensive study of these analytes indicates that the NBC has sensitivity twice that of ELISA and LOD values one order of magnitude lower.[30] Figure 4a is a typical dose–response curve obtained via the NBC with increasing amounts of analyte. As an additional validation, detecting CEA in saliva ($n = 6$) and serum ($n = 23$) samples by NBC correlated to clinical reference methods with $R^2 = 0.94$ and $R^2 = 0.95$, respectively.[30]

In the NBC, both intra-bead and inter-bead multiplexing is possible. First, beads functionalized with CEA and CA125 capture antibodies are compared to beads loaded with both. When used to construct calibration curves across the physiologically relevant range, no significant difference in signal output is seen between sole CEA and dual CEA/CA125 beads. These dual-type beads allow an even greater number of analytes to be quantified in a small NBC area.[30] Second, in a demonstration of signal specificity, beads sensitized to CEA, Her-2/*Neu*, and CA125 are exposed to each antigen individually, followed by a cocktail of detecting antibody. Signal from the nonspecific beads is <5% of signal on the analyte-specific beads (Figure 4b.)

For heart disease studies, the programmable design was exploited for the first demonstration of acute myocardial infarction (AMI) diagnosis via saliva. Immunoassays were optimized individually within the NBC before strategic panels were compiled. Serum and unstimulated whole saliva (UWS) samples were collected from an AMI-positive population (n = 41) up to two days post presentation of chest pain symptoms. These specimens and control (n = 21) samples were analyzed for cardiac-specific biomarkers with both the NBC and reference methods. Well-established methods of data interpretation including logistic regression and AUC for ROC analysis evaluated the impact critical collections of these biomarkers would have on diagnostic insight. In saliva samples, CRP, myoglobin (MYO), and myeloperoxidase (MPO) analysis on the NBC yielded AUC values of 0.85 ($p < 0.0001$); when electrocardiogram (EKG) was added, AUC values increased to 0.96.[44] For serum samples, panels of MYO, brain natriuretic protein, troponin I, and creatinine phosphokinase myocardial band achieved AUC values of 0.98, far superior to the ~0.6 AUC values typical of EKG analysis alone. The translation of these NBC measurements from the centralized hospital laboratory to the POC of an ambulance can be envisioned and is now being actively pursued.27

The bead-based NBC also may be used for genomic assays. Using beads containing a DNA capture probe (with microfluidic PCR amplification designed upstream), target DNA analytes can be identified and quantified in near real time by fluorescence changes that occur during analyte binding.[28, 45] The power and utility of this DNA detection methodology was demonstrated for the analysis of samples containing a variety of similar 18-base oligonucleotides, a length highly relevant to the burgeoning field of siRNA and miRNA research. The NBC readily measures hybridization times in minutes with point mutation selectivity factors >10,000 and LOD values of 10^{-13} M.

A PROGRAMMABLE DESIGN

The NBC can examine a wide array of analytes beyond the typical life science triad of cells, proteins, and nucleic acids (Table 2). The bead-based sensor has analyzed pH, electrolytes, short polypeptides, metal cations, sugars, biological cofactors, cytokines, toxins, proteins, antibodies, and oligonucleotides, while the membrane ensemble has separated and identified cells, spores, and bacteria.[46, 47] As such, the NBC has one of the most diverse catalogues of validated proteomic analytes of any POC system in the literature (Table 2).[48] The programmability of the NBC allows customization for medical specialties or specific disease states. In cardiovascular medicine, for example, different customized “cardiac chip” panels for acute symptoms (diagnosing chest pain, aortic dissection, pulmonary embolism), prevention (monitoring atherosclerosis), and long-term care of heart failure patients are easily created simply by changing the bead types.

FUTURE DIRECTIONS

The explosion of new health-related information coming from the genomics, proteomics, glycomics, and metabolomics fields makes it important to develop new procedures that use these scientific discoveries. There are indeed a huge number of biomarker “discovery” papers —20,000 from the cancer and 6,000 from the cardiac areas alone—yet only ~1 biomarker per

year was approved by the FDA between 1995 and 2005.[49] This slow rate of approval for new biomarkers for use in clinical practice is largely a limitation of the analyte-specific design of current bioanalytical and clinical technologies.[50]

We suggest here that researchers in the POC field take inspiration from the microelectronics arena, in which a standard operating system in tandem with modular software programs specific to a *variety* of applications serve as ideal models for future efforts in the medical microdevice area. To stimulate the medical microdevice area to take advantage of the fruits of the labor from the currently untapped biomarker discovery area, we propose there be a focus on a new figure of merit that features the number of analytes that can be measured for a single patient in a given amount of time, a model akin to the microelectronics field. There is evidence that this quotient is increasing exponentially as increasingly advanced POC devices provide more biomarker fingerprint data for each patient in a quicker period.[48]

Although diagnostic devices for electrolytes, glucose, blood gas, and cardiac testing at the POC are available, the use of such products in emergency rooms is still quite limited.[51] The poor analytical performance relative to remote laboratories, limited analyte diversity, slow development time, and higher costs have served as barriers for further expansion of these tests. Additional studies are clearly needed to convince the appropriately cautious medical community of the validity and utility of such approaches. The NBC's ability to combine measurements of cells, proteins, oligonucleotides, and small molecules on one high performance instrument that offers a more cost-effective and information-rich approach may help to usher in a new generation of diagnostic devices that can facilitate the arrival of an information rich experience to the clinical diagnostics area that has in the past been limited to the software and electronics industries.

Acknowledgments

Funding for this work was provided by the National Institutes of Health (NIH) through the National Institute of Dental and Craniofacial Research (U01 DE15017 and U01 DE017793). The authors also thank Jorge Wong for graphic assistance with Figure 1. The content is solely the responsibility of the authors and does not necessarily represent or reflect views of the NIH or the U.S. government.

REFERENCES

1. Myers FB, et al. Lab Chip 2008;8:2015–2031. [PubMed: 19023464]
2. Walt DR. Science 2005;308:217–219. [PubMed: 15821081]
3. Ouellette AL, et al. Anal. Chem 2009;81:3216–3221. [PubMed: 19368339]
4. Whitesides GM. Nature 2006;442:368–73. [PubMed: 16871203]
5. Vilkner T, et al. Anal. Chem 2004;76:3373–3385. [PubMed: 15193114]
6. Mukhopadhyay R. Anal. Chem 2009;81:4169–4173. [PubMed: 19422188]
7. Lavigne JJ, et al. J. Am. Chem. Soc 1998;120:6429–6430.
8. Goodey A, et al. J. Am. Chem. Soc 2001;123:2559–2570. [PubMed: 11456925]
9. Goodey AP, et al. J. Am. Chem. Soc 2003;125:2870–2871. [PubMed: 12617642]
10. Hunter DJ, et al. N. Engl. J. Med 2008;358:105–107. [PubMed: 18184955]
11. Lee HJ, et al. Analyst 2008;133:975–983. [PubMed: 18645635]
12. Stoeva SI, et al. J. Am. Chem. Soc 2006;128:8378–8379. [PubMed: 16802785]
13. Yager P, et al. Nature 2006;442:412–418. [PubMed: 16871209]
14. Melin J, et al. Annu. Rev. Biophys. Biomol. Structure 2007;36:213–231.
15. Holmesfarley SR, et al. Langmuir 1988;4:921–937.
16. Martinez AW, et al. Proc. Natl. Acad. Sci. U.S.A 2008;105:19,606–19,611.
17. Thorsen T, et al. Science 2002;298:580–4. [PubMed: 12351675]
18. Goluch ED, et al. Lab Chip 2006;6:1293–1299. [PubMed: 17102842]

19. Osterfeld SJ, et al. Proc. Natl. Acad. Sci. U.S.A 2008;105:20,637–20,640. [PubMed: 18162539]
20. Kim JS, et al. Anal. Chem 2009;81:5426–5432. [PubMed: 19496600]
21. Wang WU, et al. Proc. Natl. Acad. Sci. U.S.A 2005;102:3208–3212. [PubMed: 15716362]
22. Sia SK, et al. Angew. Chem. Int. Ed 2004;43:498–502.
23. Herr AE, et al. Ann. N.Y. Acad. Sci 2007;1098:362–374. Oral-Based Diagnostics. [PubMed: 17435142]
24. Davis G. Chemical Analysis (New York) 1998;148:47–76. Commercial Biosensors.
25. Madou M, et al. Annu. Rev. Biomed. Eng 2006;8:601–628. [PubMed: 16834568]
26. Rodriguez WR, et al. PLoS Medicine 2005;2:663–672.
27. Christodoulides N, et al. Clin. Chem 2005;51:2391–2395. [PubMed: 16306107]
28. Ali MF, et al. Anal. Chem 2003;75:4732–4739. [PubMed: 14674448]
29. Floriano PN, et al. Biosens. Bioelectron 2005;20:2079–2088. [PubMed: 15741078]
30. Jokerst JV, et al. Biosens. Bioelectron 2009;24:3622–3629. [PubMed: 19576756]
31. Christodoulides N, et al. Meth. Mol. Biol 2007;385:131–144.
32. Kirby R, et al. Anal. Chem 2004;76:4066–4075. [PubMed: 15253644]
33. Hanash SM, et al. Nature 2008;452:571–579. [PubMed: 18385731]
34. Wong, DT. Salivary Diagnostics. Wiley-Blackwell; Hoboken, NJ: 2008. p. 301
35. Mukhopadhyay R. Anal. Chem 2007;79:3248–3253. [PubMed: 17523228]
36. Mukhopadhyay R. Anal. Chem 2005;77:429A–432A.
37. Ligler FS. Anal. Chem 2009;81:519–526. [PubMed: 19140774]
38. Jokerst JV, et al. Lab Chip 2008;8:2079–2090. [PubMed: 19023471]
39. Weigum SE, et al. Lab Chip 2007;7:995–1003. [PubMed: 17653341]
40. Weigum SE, et al. Can. Prev. Res. 2010 in press.
41. Mandel ID. J. Am. Dent. Assoc 1993;124:85–87. [PubMed: 8445148]
42. Herr AE, et al. Proc. Natl. Acad. Sci. U.S.A 2007;104:5268–5273. [PubMed: 17374724]
43. Thekkek N, et al. Nat. Rev. Cancer 2008;8:725–731. [PubMed: 19143057]
44. Floriano P, et al. Clin. Chem 2009;55:1530–1538. [PubMed: 19556448]
45. Li S, et al. J. Microelectromech. Syst 2006;15:223–235. [PubMed: 19829760]
46. McCleskey SC, et al. J. Am. Chem. Soc 2003;125:1114–1115. [PubMed: 12553782]
47. Wright AT, et al. J. Am. Chem. Soc 2005;127:17,405–17,411.
48. Jokerst JV, et al. Nanomedicine 2010;5:143–155. [PubMed: 20025471]
49. Vitzthum F, et al. J. Proteome Res 2005;4:1086–1097. [PubMed: 16083257]
50. Anderson NL, et al. Mol. Cell. Proteomics 2002;1:845–867. [PubMed: 12488461]
51. Simonsen M. The BBI Newsletter. 2005

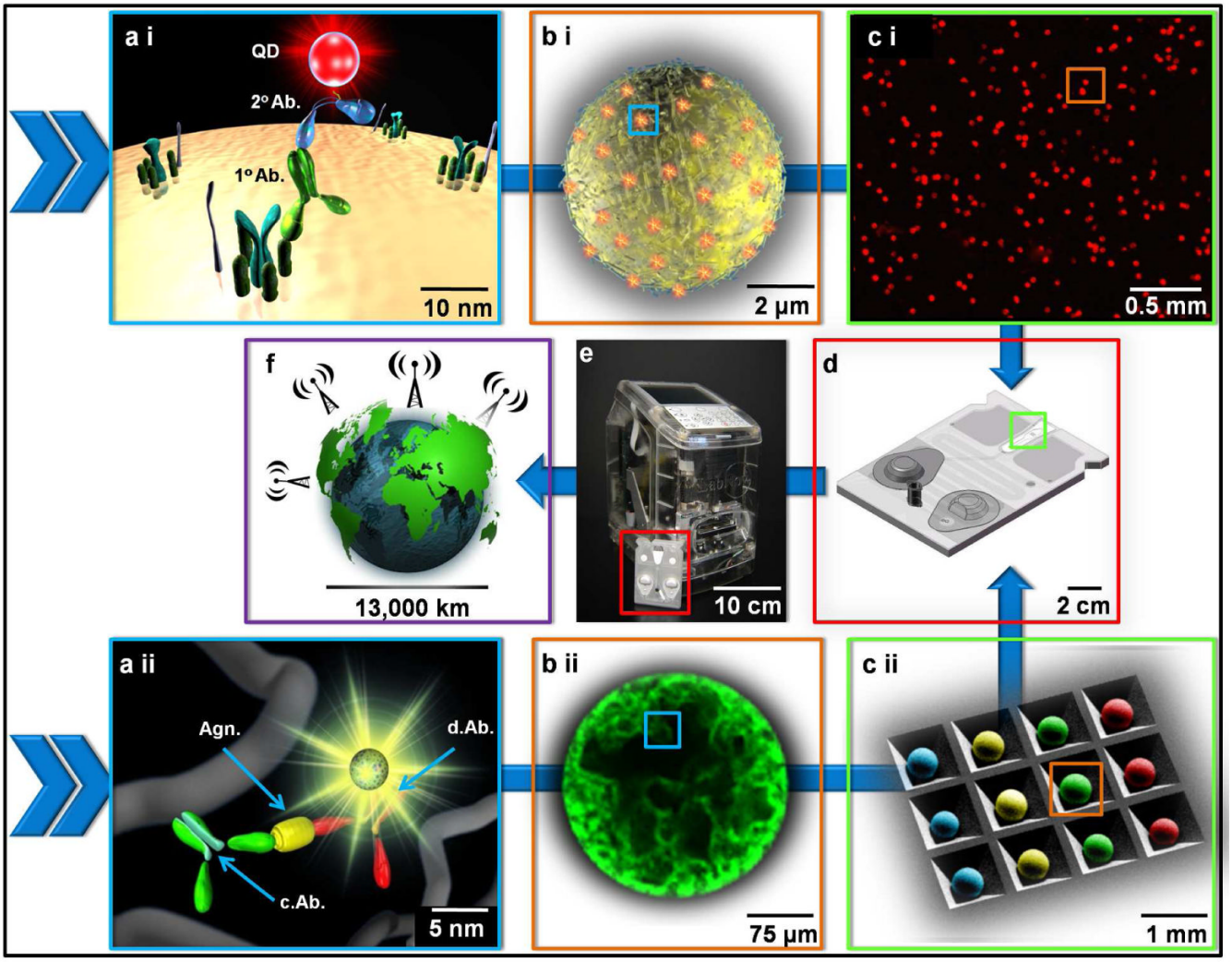


Figure 1.

The membrane-based cellular processing unit (scheme i) has demonstrated utility in cell-based assays, whereas the bead-based chemical processing unit (scheme ii) is more often used for protein assays. Analytes are labeled with a fluorophore on the nanometer scale either on the cell surface (ai) or within a sandwich assay in the agarose bead (aii). These fluorophores are densely functionalized both on the cell surface (bi) and throughout the micrometer-sized bead (bii). Arrays of cells (ci) or beads (cii) in the millimeter range form the diagnostic core of the NBC and are housed in the compact, modular labcard (d). Interface with the human user occurs via a portable, self-contained analyzer (e) with the potential to affect health on the global scale through the collection, distribution, and management of healthcare data with the aid of internet resources (f).

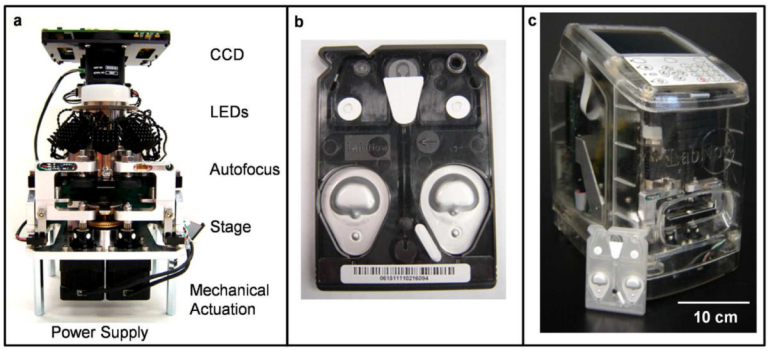
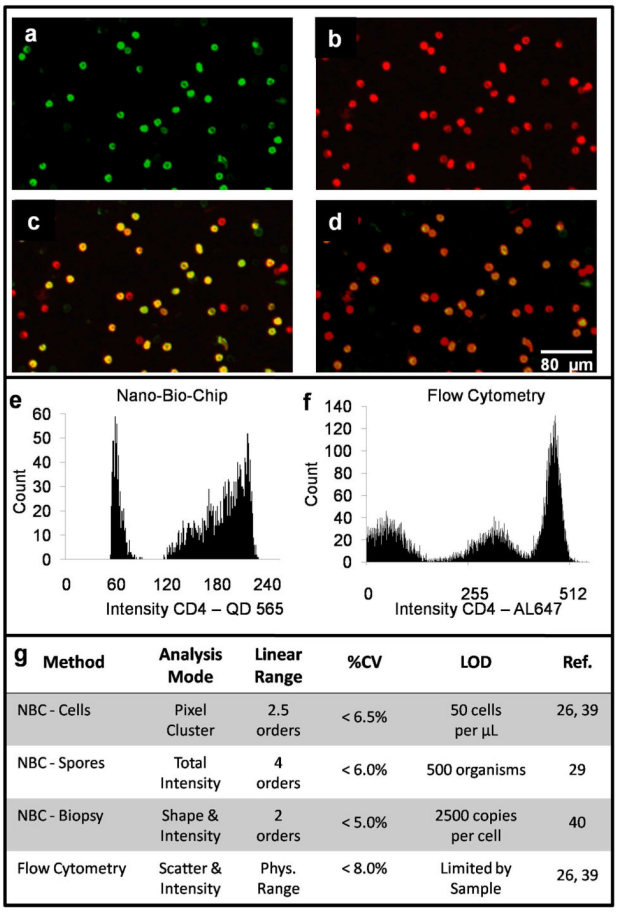


Figure 2.
Assays performed with the NBC are completed with the compact, toaster-sized analyzer currently under development by LabNow. a) Analytical core of the analyzer with optical, electronic, and mechanical component features. b) The biochemical reactions are all performed within the compact labcard. c) The assembled analyzer device and labcard create an integrated, analytical approach with dimensions conducive to POC analysis.



g Method	Analysis Mode	Linear Range	%CV	LOD	Ref.
NBC - Cells	Pixel Cluster	2.5 orders	< 6.5%	50 cells per μL	26, 39
NBC - Spores	Total Intensity	4 orders	< 6.0%	500 organisms	29
NBC - Biopsy	Shape & Intensity	2 orders	< 5.0%	2500 copies per cell	40
Flow Cytometry	Scatter & Intensity	Phys. Range	< 8.0%	Limited by Sample	26, 39

Figure 3.

Results of membrane-based NBC cellular processing. (a) CD4+ cells are green, and (b) CD3 + lymphocytes are red as observed through separate filter cubes. (c) Digital overlap of the two images illustrates CD3−CD4+ cells (monocytes, green), CD3+CD4− cells (red), and CD3 +CD4+ (T lymphocytes, yellow). (d) A long pass emission filter allows a single capture event to produce a similar image. Histograms of (e) NBC and (f) FC analysis reveal bimodal distribution in CD4+ cells and clear baseline resolution of the NBC approach. (g) Comparison of performance features within a membrane-based NBC illustrates its capacity to examine a broad range of analyte classes via different image-based descriptors.

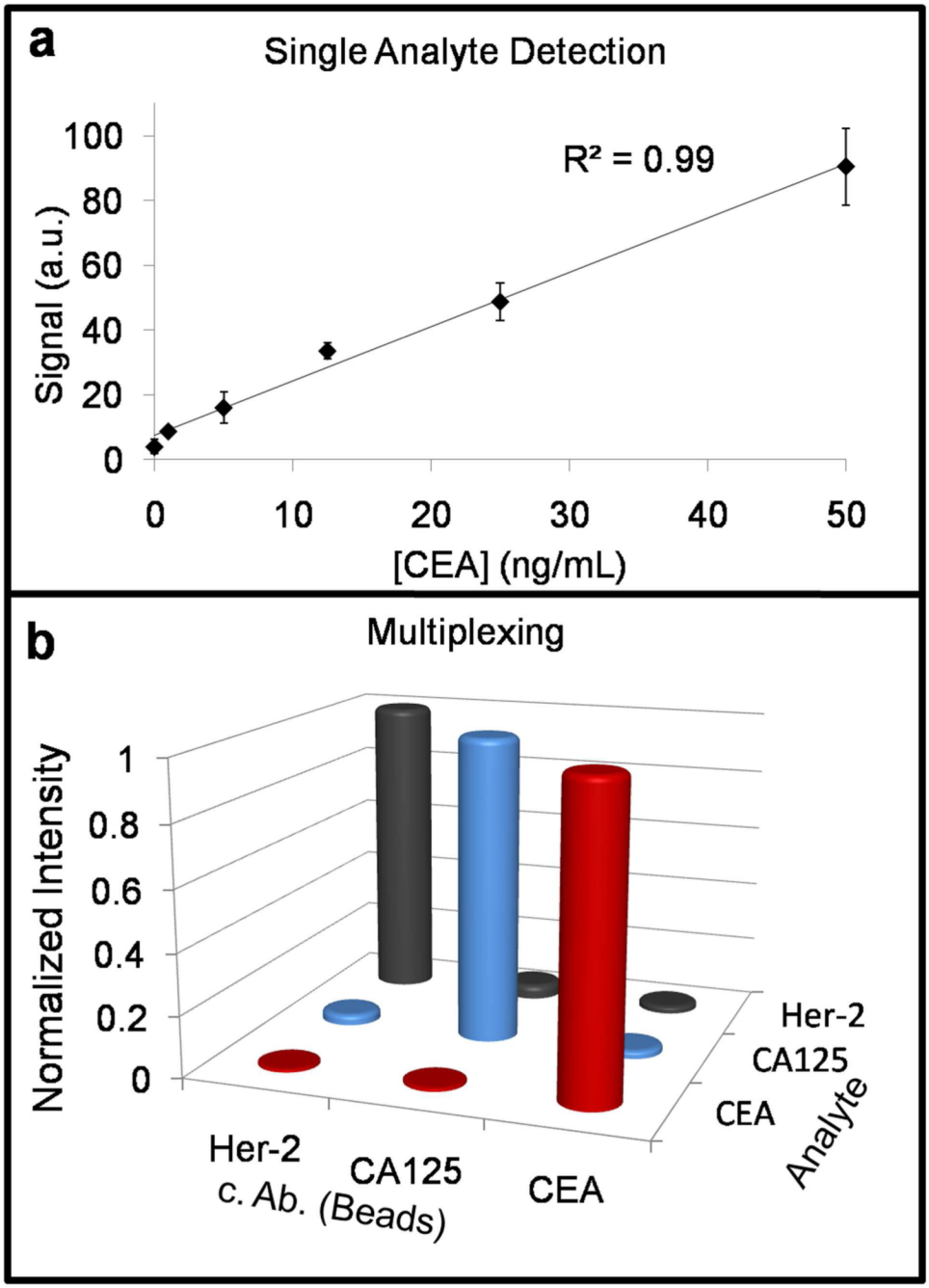


Figure 4.
a) Dose–response curve typical of the NBC. (b) Inter-bead multiplexing: low nonspecific binding between array channels specific for three different biomarkers.

Table 1

Various measurement approaches for CRP along with relevant analytical descriptors. The NBC approach (LOD = 10.0 fg/mL) is competitive with some of the most sensitive methods

Approach	Technique	Dynamic Range	Limit of Detection	Intra-Assay (%CV)	Inter-Assay (%CV)
Texas Microchip	NBC	PBS: 20 fg/mL–100k ng/mL Saliva: 10–10,000 pg/mL Serum: 0.2 -100,000 ng/mL	PBS: 10.0 fg/mL Saliva: 1.0 pg/mL Serum: 0.1 ng/mL	8%	3–10%
ALPCO	ELISA	19–150 ng/mL	0.12 ng/mL	6%	12%
Diagnostic Systems Lab	ELISA	10–500 ng/mL	1.6 ng/mL	3%	5%
Dade Behring	IN	175-11,000 ng/mL	20.0 ng/mL	N/A	4.3-6.8%
Wako	IT	50–10,000 ng/mL	60.0 ng/mL	N/A	1–11%
Roche	Particle-IT	100–20,000 ng/mL	210 ng/mL	N/A	0.6–7.2%
Abbot	Latex-IT Microparticle	50–30,000 ng/mL	N/A	N/A	6.7–12%
Diagnostic Products Corp.	IL	100–250,000 ng/mL	20 ng/mL	N/A	6.4–12%

IN, immunonephelometric; IT, Immunoturbidimetric; IL, Immunoluminometric

Anal Chem. Author manuscript; available in PMC 2011 March 1.

Table 2

From early studies with cations and small molecules, the NBC continues to find an ever-expanding role in clinical analysis. Bottom: Extended list of analytes measured with the NBC approach

Analyte Class	Examples	Range/LOD	Gold Standard	Agreement	Sample Matrix	Ref.
Sample Cations	H^+, Ca^{2+}, Citrate	2 < pH < 12 10^{-7} M	ISE	R^2=0.99	Serum, Buffer	7,8,9
Small Molecules	Saccharides, ATP/ADP	20 mM	ELISA	N/A	Buffer	46
Peptides	Tachykinin, Neurokinin	N/A	EIA	N/A	Buffer	47
Nucleic Acids	DNA-18mer	10^{-13} M	PCR	N/A	Buffer	28,45
Spores	Bacillus	500	Culture	N/A	Bioaerosols	29
Cytokines	TNF-α, IL-1ß, IL-8, IL-6	1 ng/mL	ELISA	N/A	Serum, Saliva	32
Cells	CD 2, 3, 4, 8, 19, 25, 45, 56, 69; Lymphs., Monos., Epi.	50–1,500 cells/μL	Flow Cytometry	R^2=0.98	Whole Blood, Oral Samples	26,39,40
Biomarkers	Cardiac: CRP, MPO, cTnl Cancer: CEA, CA125, Her-2/Neu	10 pg/mL CRP 0.11 pM CEA	ELISA, Benchtop Analyzers	R^2=0.99	Serum, Plasma, Saliva Buffer	27, 31, 33, 44

Assays Completed: ATP, ADP, C-Reactive Protein, Myeloperoxidase, Soluble CD40 Ligand, Monocyte Chemoattractant Protein-1, Human Serum Albumin, Troponin I, Troponin T, D-Dimer, Apolipoprotein A1, Apolipoprotein B, Tumor Necrosis Factor-Alpha, Interleukin-6, Interleukin-1ß, Transferrin, Amylase, Total human IgE, Allergen Specific Human IgE (~ 8 allergens), Brevetoxin (competitive), Matrix Metalloproteinases 8 and 9, Brain Natriuretic Peptide, Hepatitis C, Hepatits B-Specific IgG, Influenza A, p24 Antigen (proof of principle)/competitive, gp125-Specific Human IgG, Her-2/*neu*, Carcinoembryonic Antigen, Cancer Antigen 125, Ricin, α-Neurokinin, Substance P, Cytomegalovirus, TP53, Epithelial Growth Factor Receptor