BioMEMS

Technical Overview

The state of the art in Point-of-Care *in vitro* diagnostics

When considering the uses and appropriateness of various IVD methods and platforms at POC, the development process typically follows a well-trodden path: select a therapeutic or diagnostic target, identify biomarkers with clinical relevance, followed by development of a testing method. After more validation testing, that process and method drive the selection and design of the testing platform, perhaps as a lab-on-a-chip (LOC), a microelectromechanical system (MEMS), lateral flow or similar device for POC use.

Purpose-built diagnostics are an accepted approach, and the limitations are apparent

When LOC, MEMS or other chip-based or microfluidics platforms are developed for a particular set of biomarkers, a purpose-built diagnostics device is typically designed and fabricated. Change any parameter or select an alternative biomarker, and a new device or chip likely needs to be designed, built and tested, along with new protocols.

Further, in the rush to miniaturization, developers often push the limits of nanofabrication, which can create more problems than it solves. This can drive up development costs, impact manufacturing yields and affect device performance. The result of this is evidenced by the high cost of LOC, MEMS and similar diagnostic devices, where a single LOC chip costing upwards of $100 is not unusual. Understandably, widespread adoption of these devices hasn't occurred. In the case of almost all similar chip-based devices, their cost and complexity generally keep them from general use in a POC setting, especially by low-skill personnel. In fact, most MEMS are currently Research Use Only (RUO) devices.

Another common drawback of current PCR, flow cytometry and lateral flow devices is limited multiplexing, requiring multiple tests and sample-splitting to detect a group of biomarkers relevant to a particular diagnosis or therapeutic target. This adds cost, complexity, and increased potential for processing errors. In many cases, these devices do not achieve sufficient sensitivity to allow accurate quantitation at native concentrations in raw biofluid without amplification or culturing before analysis. This additional sample processing not only increases the likelihood of contamination and quantitative error but also adds significant time to results.

A significant directional shift

Instead of designing a new chip or device for each biomarker panel, BioMEMS principal scientists under the leadership of Chief Science Officer Dr. Michael J. Pugia developed a universal, standardized, biomarker-agnostic, biofluid-agnostic hybrid MEMS sensor array and sample processing protocol to detect and quantify biomarkers with previously unattainable sensitivity and specificity. The proprietary, patent-pending hybrid MEMS design can be manufactured cheaply in the millions as a "blank" MEMS sensor array to which extremely small amounts of up to ten (10) different reagents can be applied in a secondary, proprietary process to create a bespoke test or test panel quickly and cost-effectively.

Over the years, the pool of commercially available, clinically relevant and compliant immunoassays, antibodies, affinity agents and nucleic acid probes has proliferated, exceeding 200,000 by some estimates. There is really no need to re-invent many of them, thus compressing test development, compliance, and speed to market, as the BioMEMS platform can integrate and actualize most, if not all, such chemistry. In all, BioMEMS can demonstrate economies of scale as well as predictable quality and yields for a multiplexed test cartridge using a single biofluid sample at point of care.

The design of the hybrid MEMS sensor array also exponentiates the sensitivity and specificity of commercially available assays, antibodies or probes, which after a proprietary optimization process, have been tested to achieve 5 picomolar or better quantitation of target biomarkers in 1 microliter biofluid aliquots – or, roughly 10x more sensitive than other POC devices.

This novel approach represents a disruptive change in POC diagnostics. Now, high-need, high-complexity tests and test panels can be quickly developed and manufactured with quality and consistency at exponentially lower cost.

Competitive Analysis

The BioMEMS approach addresses several challenges with existing biofluid sampling and detection methods, among them immunoassays, electrophoresis, spectrophotometry, immunomagnetic assays and others.

Immunochromatography – an industry-standard approach to IVD – generally lacks the sensitivity required for most assays in infectious disease, cardiac disease, cancer, and endocrinology to name a few. While currently available immunochromatography techniques can occasionally achieve nanomolar detection levels for a limited number of analytes, the signals generated at those levels are miniscule and reproducibility of the signal is a real issue.

Genetic or DNA-based tests such as polymerase chain reaction (PCR) tests are useful in determining the presence of a specific pathogen or some portion of its DNA but is not applicable to biomolecules such as hormones, cytokines, chemokines, kinases and other proteins or peptides of interest. PCR tests provide for a sensitive and specific solution but suffer from contamination due to nucleic acids in complex biofluids that can limit amplification for target copies of less than 100,000 particles/milliliter concentrations.

Lab-On-A-Chip (LOC), older microelectromechanical systems (MEMS) and silicon-based microfluidic devices typically incorporate PCR, immunoassay, or flow cytometry into their detection method or quantification, often with limitations and compromises, and usually at much higher cost when compared to the BioMEMS platform. Many of these devices rely on optical methods such as fluorescence, luminescence or adsorbance, which must undergo routine calibration. Additionally, the miniaturization potential of optical systems is limited and complex, which can add significant cost to their development and restrict their use to a benchtop setting.

Wet or dry clinical chemistry tests are abundant and inexpensive. Most large analyzers found in clinical labs use some form of reagent chemistry to tag, titrate or otherwise detect various biomarkers. Glucose or A1C test strips utilize an electrochemical reaction and a meter to interpret the results. Dry reagent chemistries make up the majority of low-cost options and use dry reagent pads exposed to

biofluid samples, usually resulting in a shift of color. Dry reagent or dipstick methods are generally binary in results reporting or offer only a limited range of accurate quantification.

BioMEMS does not intend to displace common, ubiquitous test panels or protocols, instead focusing on new and novel test panels, or high-need, high-complexity panels that can benefit from the speed, sensitivity and specificity the BioMEMS platform can deliver at point-of-care or self-administered at home, at work or remote locations. Please see attached competitive exhibit.

BioMEMS Design Brief

The BioMEMS test platform is built around an electrochemical immunoassay system with the unique capability of processing complex biofluid samples rapidly using size exclusion filtration passaged through a proprietary microelectromechanical sensor array. A single-use capture and sensing cartridge is used in combination with a handheld analyzer which activates the cartridge and ports raw results via Bluetooth to a sophisticated normalizing and analytics app that can be downloaded to most smart devices. Results are typically available in less than 5 minutes of obtaining the biofluid sample.

The BioMEMS platform is designed to surpass the challenges and limitations of currently available technology to work with any complex biofluid. It can be configured to detect a broad range of targets, from individual pathogens to small molecular entities. The single limiting factor is the availability of an affinity agent that binds or interacts with the biomarker or pathogen of interest. This means that hundreds, if not thousands, of unique assays can be created from the current available pool of 200,000+ assay, antibodies, probes and reagents using the same capture and sensing technology.

When a test cartridge with biofluid sample is inserted into the analyzer base, the cartridge captures concentrates, and purifies analyte molecules by affinity surface capture on the micro-filtration sensor membrane. The surfaces of individual microwells are chemically modified to allow rapid removal of background signals while at the same time concentrating the analyte(s) of interest with antibodies or other affinity agents on the sensor surface. The removal of background signals from the desired analyte gives the immunoassay reagents the ability to operate under ideal conditions and to generate clean results without interferents. This simple and efficient process does not have the legacy problems of competing methods, where the analyte fails to bind to separation surfaces consistently, resulting in different results from the same sample.

Engineered to be small and disposable, the test cartridge is designed so that the user does not touch or interact with chemistry or affinity reagents, or the biofluid sample itself. The unique configuration allows for filtration and detection simultaneously, effectively reducing assay run time. The design allows for ultra-high sensitivity electrochemical assays within a single microwell, each such microwell representing an individual immunoassay result. Ultimately, this requires far smaller amounts of antibody reagents in the cartridge to achieve a quantitative result that is faster and more accurate than other IVD products. Furthermore, confirmatory sampling is not necessary as the cartridge is designed for the captured and purified analytes to be released on demand for confirmatory testing by orthogonal methods (liquid chromatography mass spectrometry, PCR, etc.)

By combining high sensitivity and analyte specificity with microscale dimensions, the cost of manufacturing the test cartridges is greatly reduced. The miniaturized, disposable test cartridge

design also drives down operating costs, as the system uses an internal standard for live electrochemical calibrations in each sample, thereby successfully eliminating maintenance that comes with the current state of laboratory-scale or POC equipment.

The BioMEMS platform is intended to displace quantitative and qualitative assays at point-of-care (POC) specifically where conventional immunoassays or chemistries lack the sensitivity or specificity needed to measure pre-clinical to clinical levels of biomarkers, toxins and pathogens at extremely low concentrations in saliva, urine, blood or any other complex biofluid. This would be especially applicable to the most difficult and esoteric targets where sensitivity and specificity are most vital i.e., infectious disease, chronic disease monitoring, cardiac testing, cancer testing and endocrinology.

Some manufacturers and clinicians believe that poorly performing diagnostic systems can generate false positives or negatives at a rate of 30% or greater. The cost of those false results, either due to low manufacturing quality, sensitivity or specificity greatly increases the time to resolve a specific disease, confuses both patients and clinicians, and generates poor health outcomes overall. A surprising number of commonly used POC and laboratory methods are unable to achieve both analytical sensitivity and specificity in range of 95% to 99%.

The future is equally compelling. BioMEMS has already planned and is developing continuous improvements to its core technologies. Intellectual property protection of all assets is vital to the success of the endeavor, as is the continued drive toward technological leadership in the IVD space.

BioMEMS has recently filed for patent protection of enhanced mechanisms for easy sample loading, more efficient delivery of liquid reagents within the cartridge, more effective removal of affinity captured analytes, a safe sample archival mechanism for same sample re-testing and methods for manufacturing and calibration.

The true power of the BioMEMS platform is not only the superior sensitivity and specificity, but also the ability to develop customized assay panels according to customer requirements and market demands without the need to design and build new chips, new hardware or develop new protocols, exponentially driving down costs and development time. A streamlined development process has been crafted so a partner/customer can go from concept to reality in months, rather than years.

Platform Advantages Specific to Challenging Environments

Although not immediately obvious, much of today's IVD tests and analyzers rely on gravity and a stationary, upright position to perform, especially if there are tanks or reservoirs for reagents, washes, enzymes, etc., much less the actual sample capture itself, whether by syringe/vacuum tube, cups or finger-sticks.

As product development unfolds, BioMEMS principals intend to design the sample capture, isolation, detection and archiving to be independent of physical orientation and to develop sample capture interfaces for samples as diverse as wound fluid, tears, mucus, cerebrospinal fluid, etc. in addition to the more typical sampling of saliva, urine, whole blood, plasma and serum.

Each test cartridge contains all the necessary chemistry to perform one or more tests, in most cases using sample volumes of less than 1 microliter. Once inserted into the analyzer, the vacuum created

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by the analyzer provides the impetus to move the sample through the various stages within the test cartridge, as well as receiving and amplifying the electrical impulses generated by the sensors as the electrochemical reaction ensues within the test cartridge.

The key working components of the entirely self-contained test cartridge are fabricated at microscale, including the filling of micro-reservoirs with wash and affinity agents, all protected by a robust protective matrix that locates and fixes the position of the various micro-reservoirs, microfluidic channels and microwells. The result is a robust, largely shockproof test cartridge. The construction matrix of the analyzer is similar.

The BioMEMS platform has addressed the challenges of complex IVD processing in challenging environments, ranging from less-than-hygienic environments in remote settings to low-gravity/high-inertia conditions.

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PCR = Polymerase Chain Reaction **FC** = Flow Cytometry **LOC** = Lab on Chip **MEMS** = Micro Electromechanical Systems **IC** = Immunochromatography

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Technology	Issues/Limitations	Companies	BioMEMS	Competitive Advantages/Features
PCR	• Time to result (min. 30 minutes) • Costly reagents • Susceptible to background contamination • Limited multiplexing without splitting samples • Measurements are limited to DNA/RNA -based targets	BINX QuantuMDx Biocartis Asuragen	BioMEMS	• Rapid assay times (approx. 3 minutes or less) • Extreme sensitivity without background contamination • Simple multiplexing • Easy, one -step format
FC	• Limited multiplexing • Can't isolate specific cell(s) for DNA/RNA analysis • Prone to clogging due to small capillaries, difficult to calibrate, and must constantly make new combinations of labels	Ativa Medical PixCell	BioMEMS	• Selective cell(s) capture with the ability to release and conserve cell(s) for further analysis • Able to do electrochemical reactions for chemistry and response assay • One common universal labeling system
LOC	• Expensive to manufacture and sell • Do not provide for a single -use application • Not for at -home use • Requires justification of highly multiplexed panels	Micronics Abionic ChipCare	BioMEMS	• Low -cost sensor • Allows sample to be tested at POC and then sent to a laboratory for confirmation if needed • Can perform high throughput IA in the laboratory with MS detection
MEMS	• Research Use Only (RUO) equipment • Not designed for processing complex samples in a handheld -type device • Unable to provide solution across the entire IVD market	Philips Atomica IST	BioMEMS	• Spans OTC, POC, and Laboratory IVD markets in simple, CLIA waivable format • Meets reagent cost basis for the IVD market while eliminating additional sampling • Provides immediate results for action
IC	• Qualitative results – YES or NO • Limited multiplexing, only 2x • Does not isolate and release cells or antigens • Costly and temperamental reagents	LamdaGen Genalyte Quidel	BioMEMS	• Multiplex or 10+ analytes per cartridge • Quantitative, lab -quality results • Isolation and release of antigens for conservation, further study • Isolation of cells for genetic confirmation

General advantages to BioMEMS platform technology:

- Test cartridge and chemistry provide for the conservation of the test sample that can be stored and/or tested in the future.
- Rapid results that eliminate any sample/target amplification due to the system's ultra -sensitivity
- Built-in correction/calibration allows for biofluid agnostic samples without having to include correction factors (i.e., hydrati on status for saliva, etc.)

Additional competitive analyses by company and by platform are available upon request.