Tiny but Powerful Tools for Sample Prep

Host of Technologies Born for Biodefense Improve Wide Range of Industry Workflows

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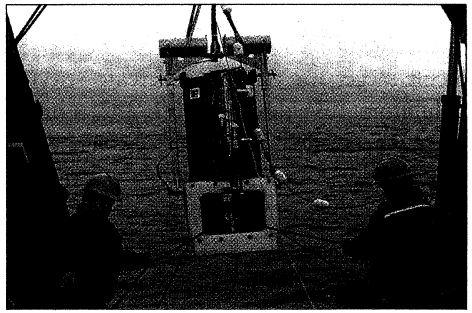
Biological research instrumentation is getting smaller and smaller as genetic and biomarker analysis moves out of the laboratory and into the clinic or the field. Smaller technologies now available include handheld immunoassay strips, flow assays, PCR systems, and mass spec equipment, but sample-prep hasn't always kept pace with the latest advances.

Centrifuges, chromatography columns, spectrometers, and other sample-prep tools are not quite so portable yet. Not surprisingly, the Knowledge Foundation's "Sample Prep" meeting held earlier this month in Baltimore was packed with presentations featuring small and sleek sample-prep technologies.

Microfluidics offers one way around the sample bottleneck, automating and miniaturizing the same sample-prep steps users would carry out at the bench. Other approaches seek to skip the prep step entirely through direct detection.



Engineers from the Monterey Bay Aquarium Research Institute deploy Spyglass Biosecurity's Environmental Sample Processor to monitor algal blooms in Monterey Bay.

InnovaPrep (innovaprep.com), which was formed last year to commercialize sample-prep technology developed at **AlburtyLab** (www.alburtylab.com), is trying to close the gap between sample-prep technologies and runaway miniaturization of biodetection technology. "We feel that what is missing in that trend and in the world is, of course, the sample prep," said CEO David Alburty. "We think of it as a link between the real-world sample size and the microliter world that those systems operate in. A macro-micro interface."

In developing its sample-concentration system, the InnovaPrep HSC-40, for the biodefense industry, AlburtyLab sought to address issues associated with collecting samples in the field. Although many detection devices are now small enough to be portable, even handheld, the size and dilution of many samples in nature remains a problem.

Taking 5 uL of water from a lake, for

[illegible] phenotypes and genotypes, targeted human subpopulation models can be employed early in the discovery and toxicity screening processes.

The pharmaceutical industry requires large numbers of purified cell types for screening candidate molecules for efficacy and unintentional toxicity, and the industrialized use of terminal cell types derived can severely restrict their pluripotency and drastically reduce the numbers of subsequently differentiated healthy cells.

Furthermore, while producing terminally differentiated cell types from stem cells using embryoid body (EB) and directed differentiation techniques are well known, the efficiency with which these methods produce terminally differentiated cells is highly variable; a [illegible] develop processes that are both scalable and standardizable for both iPSC maintenance and differentiation.

Cellular Dynamics International's (CDI; www.cellulardynamics.com) iCell™ Cardiomyocytes are human iPSC-derived cardiomyocytes that possess expected cardiac characteristics, form electrically connected syncytial layers, and exhibit expected electrophysiological properties. The primary production constraint of iPSC husbandry was eliminated by developing a culture system that uses standard single-cell splitting techniques and small molecules to minimize operator-specific effects.

iPSC culture scalability was incorporated into the process by building the cell culture system in a parallel fashion to enable the production of billions of iPSCs through the use of CellSTACK® culture chambers (**Corning**).

Differentiation of iPSCs into iCell Cardiomyocytes is built on CDI's platform that utilizes recombinant genetic engineering and antibiotic selection. Prior to iPSC clonal expansion, genes encoding antibiotic resistance and an optional marker under control of a cell-type specific promoter (pan-cardiac for iCell Cardiomyocytes) are introduced into the iPSCs through homologous recombination.

After curation and quality control (QC), the iPSC clone carrying the selectable marker is expanded using iPSC maintenance procedures, harvested, and placed into the directed differentiation protocol of choice. Subsequent to differentiation initiation, the cultures are exposed to the selection agent to leave a pure, targeted cell population.

In the case of iCell Cardiomyocytes, the directed differentiation method produces cardiomyocyte purities greater than 50%, while antibiotic selection subsequently increases this purity to approximately 100%, a level that is necessary to ensure that the observed experimental outcome is due to an effect on cardiomyocytes rather than noncardiac "contaminating" cells.

This process, as currently practiced at CDI, is capable of meeting the foreseeable demand for purified iPSC-derived human

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The electrokinetic properties of the device can be customized for the sample. "We can optimize the AC frequency or amplitude to attract certain cells based on the dielectric property of the cells and also the size. It also depends on the connectivity of the specimen," said Vincent Gau, Ph.D., CEO and president.

For example, when processing urine samples, the impedance of the sample will vary depending on whether the patient has had a lot of water to drink. Likewise, varying levels of protein in the blood will affect the impedance of the sample. Each assay begins with an impedance-matching step to select the correct current for the sample. "We identify the electrokinetic conditions to do the specific test we want to do," Dr. Gau added.

Nanopores

Liviu Movileanu, Ph.D., assistant professor in the structural biology, biochemistry, and biophysics program at Syracuse University, talked about his group's work with nanopores at the meeting. Using a combination of techniques from nanotechnology, biomolecular engineering, and surface chemistry his team is developing a chip platform for analysis of biomolecules. The sample-prep approaches previously discussed all depend on miniaturizing, automating, or simplifying the process. Dr. Movileanu said that sample prep can be effectively bypassed using an extremely sensitive detection method based on nanopores.

Natural ion channels that transport charged molecules through a potentiated membrane inspired the concept behind Dr. Movileanu's research. Increasing understanding of the mechanisms of ion channels, and the ability to measure the current running through them, have enabled scientists to replicate them in the laboratory by creating nanometer-scale holes in a silicon nitride membrane.

When placed in an electrolyte solution with voltage across the membrane, these holes behave similarly to a natural ion channel. More importantly, the current measured when an analyte passes through a nanopore positively identifies that analyte—a technique called stochastic sensing.

"This is a technique for probing very minute, small quantities of biologic material, in this case, proteins or nucleic acids. It's called stochastic sensing because each molecule interacting with a single nanpore will cause a current blockade. The nature of that current blockade is stochastic. The technique allows quantification, as well as identification of the analyte," said Dr. Movileanu.

Applications for stochastic sensing include DNA sequencing and protein detection. For example, it's possible to modify the nanopores for studying aptamers. Then, when the proteins bind to the aptamers, they create a current blockade that detects the presence of the proteins.

Microscale fabrication plays an important role in this year's crop of sample-prep innovations. Smooth surfaces, high-tech materials, and precision design make it possible to fit an entire workflow on a chip, cartridge, or handheld device. Innovative approaches to filtration and concentration can bridge the gap between the real world and the microworld on the chip.

Although the end-user applications can be extremely different, sample-prep technologies overlap significantly between environmental, biodefense, and medical research fields. For this reason, biological researchers have benefitted tremendously by investment in sample-prep technologies for biodefense applications. GEN