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# Novel Applications for Sample Preparation

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## Tool Providers Launch Innovative Products to Meet Researchers' Protein-Related Demands

Simplification, automation, and disposability were the key themes at Knowledge Foundation's recent "Sample Prep" conference in San Diego.

Speakers indicated that researchers' quest for "the next big thing" is accompanied by a push among tool developers to create the tools to help them advance that endeavor. To that end, presenters outlined advances in sample preparation, highlighting new and emerging devices that streamline workflow, increase portability and disposability, combine preparation steps, and allow multiple analytes to be purified from a single sample. A universal sample-preparation process for protein, DNA, and RNA designed by Integrated Nano-Technology, (INT) meets that latter goal.

As a component of the Palladium System, sample preparation is one part of a new, automated, approach for field testing and point of care. It uses sonication and magnetic separation techniques to clean and concentrate the DNA sample for identification. INT has developed a novel fluidic system to automate the entire process. The fluidic cartridge includes a unique rotary valve that minimizes consumable costs.

The company's goal, according to D. Michael Connolly, Ph.D., president, was to automate sample preparation and to develop a universal application. "When we set up the sonicator, we modified it to work with a wide variety of samples such as blood, tissues, and whole insects." A sample containing spores, tissues, and viruses can be purified in Palladium, which allows efficient recovery of target molecules from difficult samples without compromising the other analytes.

This ultrasonication method uses glass beads to break the cells. The approach for protein is gentler than the approach used for DNA, but it is basically the same. The objective is to maintain the protein in its native form, without exposing it to detergents or heat, which are used in the DNA-purification process. "We've also made our own nanoscale particles. They bind quite effectively to the DNA, and we get very high yields," Dr. Connolly said. Recovery rates for DNA are "better than 95 percent, and the particles also bind protein well."

INT is engineering a device now, with development at the breadboard stage. Dr. Connolly expects the final version to measure about 6" x 6" x 4" and to operate on rechargeable batteries similar to those of a laptop.

The need for smaller sample-preparation devices is driven by the appearance of small-scale sequencing, which needs correspondingly small sample-preparation devices, as well as by diagnostics and analytical applications. Uses include biothreat analysis and veterinary diagnostics.

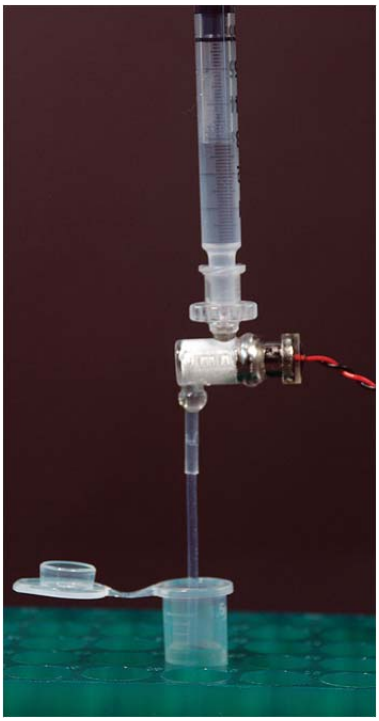
### Advanced Combinatorial

The point-of-care market is the target for Receptors, which is bringing its Affinity-by-Design approach to market now. Based upon CARA™ ACTIVEcapture™ technology, it offers a single solution for sample preparation that can be used in the detection and diagnosis of microbial contamination and infection control.

"There's a huge need for organism isolation," Robert E. Carlson, Ph.D., president and CEO, says. Traditionally, researchers had a choice between very generic methods, like centrifugation, and very specific methods, like antibody affinity capture. "There was no middle ground. ACTIVEcapture sample-preparation products bridge the critical cost versus efficiency market gap."

CARA ACTIVEcapture technology allows multiple targets to be extracted from a single column, magnetic bead, or fiber support. "It combines the strength of an antibody affinity capture with the scalability of organic chemistry." CARA is a combinatorial artificial receptor approach that is designed to adapt to a broad spectrum of applications by using a small library of subunits. This approach is similar to the strategy used by amino acids to create a diverse body of binding interactions.

"A few molecular building blocks are covalently immobilized to a support surface, which displays the region, stereo, and spatial relationships possible for each combination without the need for discrete synthesis." Combinatorial arrays use multiple wells to screen against hundreds to thousands of combinations.



According to Claremont BioSolutions, its PureLyse kits provide ultrarapid lysis and DNA extraction.

In contrast, with CARA ACTIVEcapture technology, "We get it all, so we screen against as many as 10,000 combinations at once. Although this display is heterogeneous at the molecular scale, it is statistically reproducible on the macro-scale. CARA provides binding space diversity and polyvalent spatial display…in a simple, economic, and flexible format."

### Disposability

Simplification and disposability are the key attributes of PureLyse® according to Claremont BioSolutions. This device facilitates both protein and DNA sample preparation, Bruce Irvine, CTO, explained. "It is fast and only has two steps. It is very conducive to integration into the field or point-of-care devices."

PureLyse uses a disposable micro-motor and specially designed impellor to lyse cells. As the sample lyses, nucleic acid or protein binds to the lysing particles. Because it flows through a chamber during lysis and binding, a wide range of volumes can be processed, with DNA binding up to 10 µg. "It works the same for protein and for DNA," Irvine said, and produces results with better than 95% purity.

"Alternatively, our OmniLyse® cartridge can be used for cell lysis only, and the lysed sample can then be delivered for further processing. The lysing particles can also be functionalized for affinity capture of proteins." For example, HIS-tagged proteins bind to a nickel column in the HisExpress™ version. Two additional versions are planned for release this spring.

Irvine reported a strong yield of protein using the PureLyse system, and noted that proteins can be extracted and purified in less than 10 minutes. (For DNA, the process requires less than three minutes.)

This project grew from a defense contract, Irvine explained. "An efficient, disposable method to lyse cells was needed." Claremont BioSolutions is also developing a benchtop version of PureLyse, called the RapidLyser™ with higher shear force and flow through configuration.

Streamlined Prep

Stratec Molecular, until recently known as Invitek, is simplifying sample preparation with a chemistry "that allows us to combine some of the separation steps, creating, essentially, a master mix," explained Len Vanderbosch, director of sales and marketing. Known as RTP® (Ready-to-Prep) technology, "this is an up-front solution to purifying quality nucleic acids for analysis. Our nonchaotropic chemistry prevents degradation of enzymes so samples can be prepared in fewer steps. Add the sample and you're ready to go."

"The technology is for blood and blood-related products, but we have kits for a variety of different samples." These kits create a more user-friendly approach to sample preparation which, he explains, is particularly important for molecular diagnostics labs in which researchers often have limited sample-preparation experience.

RTP technology reduces error rates by minimizing human handling. "Our customers say the results equal those of traditional methods, but that this approach fits their workflow better. It doesn't necessarily reduce preparation time, but streamlines the sample-preparation process."

FFPE Samples

Clinical tissue samples represent a comprehensive source of material for analyzing protein-expression patterns associated with diseases such as cancer. Millions of FFPE tissue sections are stored around the world, representing a valuable and extensive collection of samples for biomedical research. While formalin-fixation is the standard for preserving and archiving biological material, FFPE samples can be challenging to work with, in particular when analyzing proteins. Without further processing, the formalin crosslinking between proteins that occurs upon sample fixation means that the starting material is unsuitable for proteomic studies, explained Peter Porschewski, Ph.D., senior scientist at Qiagen.

Qiagen offers a range of solutions for processing FFPE samples and performing subsequent assays, including those used in protein biomarker research. "The Qproteome FFPE Tissue Kit provides an optimized protocol that uses innovative chemistry to extract total protein from FFPE tissue sections. After deparaffinization, the sample is treated with a buffer that reverses formalin crosslinking, releasing the protein molecules. Then, after preparation, the proteins can be used for downstream applications such as mass spectrometry or Western blotting."

Additionally, Qiagen has developed an alternative to formalin fixation for preserving tissue samples. "Recently, we successfully demonstrated that PAXgene, a new formalin-free tissue fixative, allows the extraction of nondegraded and immunoreactive proteins ready for subsequent downstream applications." After storing treated samples for a few days, they can be embedded in paraffin, similar to traditional treatment. "PAXgene is a new technology that has the potential to become a novel fixative for modern pathology, enabling extensive proteomics studies on clinical tissues."

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Protein Removal

At EMD Millipore, Sébastien Ribault, Ph.D., head of predevelopment-technology-collaboration, biomonitoring R&D, lab solutions, detailed a nucleic sample preparation kit called MilliPrep. "We're not developing protein sample preparation with it. We're removing protein from the sample." This kit, he said, is a response to a sample-preparation technology that was time-consuming, generated false positives because of the steps required for sample preparation, and was not adapted for the molecular biologists' workflow.

MilliPrep fully integrates the sample-preparation steps within a single, three-step, closed system that combines membrane-based filtration and magnetic bead nucleic acid purification.

To separate eukaryotic cells from mycoplasms, for example, users pipette the sample into the upper chamber of the device and place the device into a centrifuge, where the eukaryotic cells are retained on a membrane between the sample chamber and the capture chamber. "In this step, 99.9 percent of the protein and other contaminants are removed," Dr. Ribault said.

Mycoplasma are trapped on an asymmetric membrane, and fluid is drained into a waste tank at the bottom of the cartridge. "The remaining 0.1 percent of contaminants is removed with the fluid." Then, a lysing reagent is added. The sample is incubated and then centrifuged again to recover targeted mycoplasma nucleic acids.

According to Dr. Ribault, "The process eliminates false positives. It processes 20 mL volumes, which are 20 times higher than traditional column methods. Hands-on time is less than 30 minutes for ten devices."