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January / February 2013
VOLUME 2
NUMBER 1

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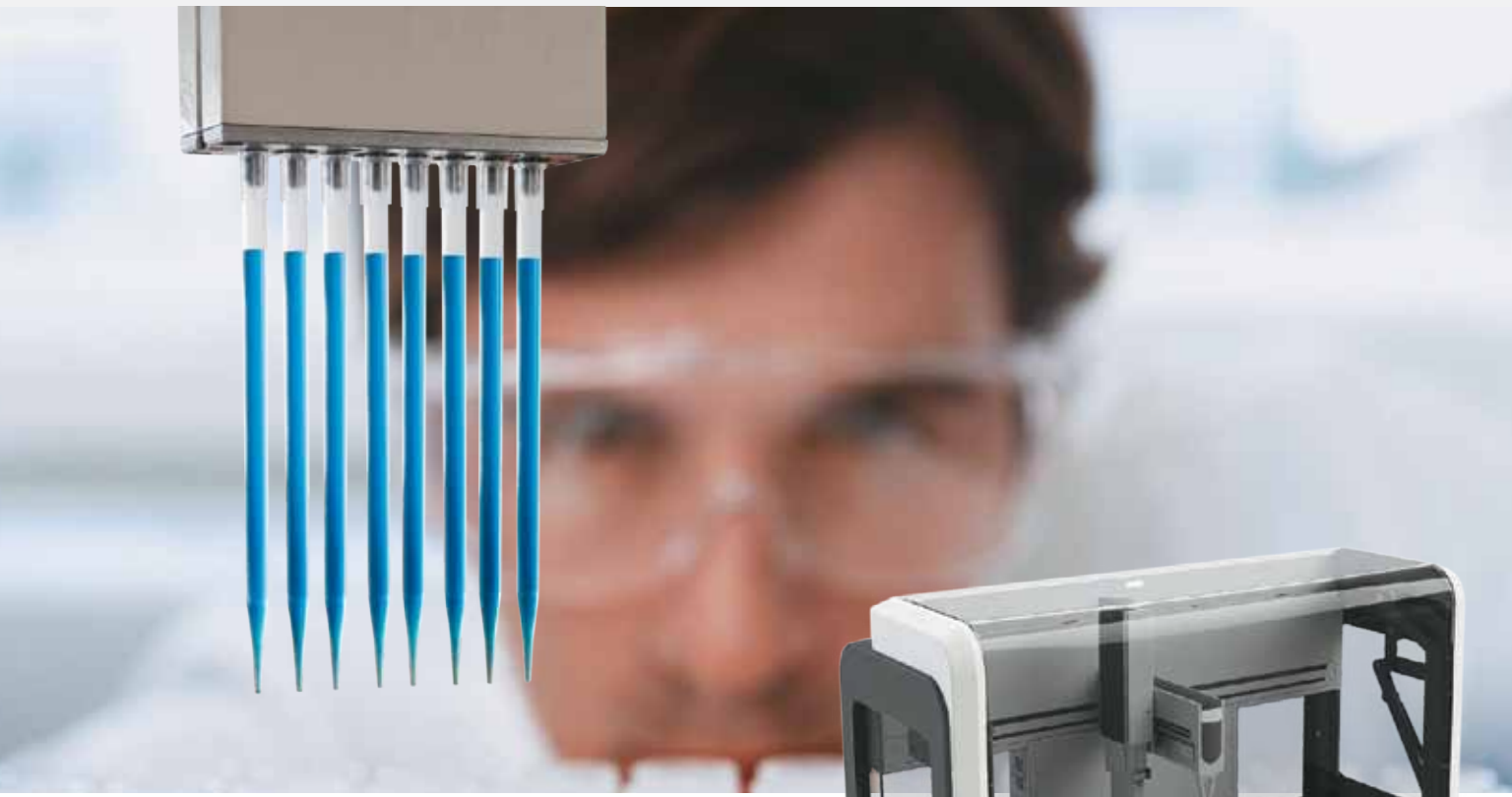

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INSIGHTS ON AUTOMATED SAMPLE PREPARATION

All articles by **Angelo DePalma, Ph.D.**

INSIGHTS
LAB TECHNOLOGY BUYER'S REPORT

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CONSISTENCY, ECONOMY, ERROR REDUCTION, AND FREEDOM FROM ROUTINE ARE COMMON DRIVERS

Sample preparation ("prep") is a tedious, time-consuming task but a necessary part of nearly every analytical workflow, regardless of industry or laboratory type.

Sample prep involves collecting, treating, and manipulating a physical substance before subjecting it to some operation, usually instrumental analysis. Some samples, like pure liquids taken from reagent bottles, hardly require preparation at all. Generally speaking, the intricacy of the preparative workflow is the product of the sample's complexity, the analytic specificity, and the ability of the instrument to discriminate from nontarget substances within the sample.

SPE Extraction System / SmartPrep™ / Horizon Technology / www.horizontechinc.com

A typical manual sample preparation workflow consists of gathering labware and reagents; calibrating measurement, delivery, and analytical systems; preparing solvents and reagents; recording relevant identifiers (lot numbers, expiration dates, weights, concentrations); labeling containers; weighing; calculating; filling; obtaining standards; and completing physical/mechanical operations such as filtering, grinding, or sonicating. Each of these steps involves multiple operations on its own. For example, glassware must be cleaned, dried, and moved around the lab, while standards need to be created, tested, and rendered into usable form through dilution, titration, and dispensing. An additional layer of recordkeeping and compliance applies to regulated laboratories.

The more complex and numerous the samples, the more critical become informatics, sample tracking, and knowledge handling. Most automated sample prep systems are connected to a laboratory information management system or are accessible through an electronic laboratory notebook, but often these require a level of "middleware" intermediary software to enable communication between system and computers.

Automatic Liquid Sampler / 7693A / Agilent Technologies / www.agilent.com

High-caliber analysis modes such as GC- and LC-MS have raised the quality standard for sample prep. As a result, laboratories view sample preparation as a bottleneck in terms of cost and worker hours.

Yet according to estimates from Agilent Technologies (Wilmington, DE), 70 to 80 percent of prep work is still performed manually. Given the concentration of automation in very high-throughput venues, it is safe to say that close to 90 percent of all labs still engage in sample prep. Reasons for not automating include acquisition and operating costs, system complexity and steep learning curves, and perceived lack of system reliability and support.

"Users expect the same robustness, usability, and uptime from sample preparation as from their analytical instruments," says Peter Mrozinski, product manager for workflow automation at Agilent. "They believe that constant tweaking at the low end and steep learning curves at the high end defeat the purpose of automation."

tasks. Very high-throughput genomics, proteomics, and medical laboratories would not exist without automation. These markets are adequately served by large, high-end systems costing hundreds of thousands of dollars. But as the number of samples decreases, nagging issues persist about learning curves, fear of automation, cost, lack of familiarity with automated workflows, and ignorance of what is possible. In other words, automation is not always an easy sell.

"Automation has recently shifted from screening toward sample preparation."

Lab automation developed from the growth of high-throughput screening in the pharmaceutical industry and then was revived by the Human Genome Project. Automation has recently shifted from screening toward sample preparation, which caused the trend away from large, complex, integrated systems to smaller, more compact, dedicated workstations.

In a review published in *Bioanalysis* (2011; 3(13), 1415–1418, Jim Shen of Merck Research Laboratories (Summit, NJ) writes that "the key to improve throughput for sample preparation in a modern laboratory is to attack any bottlenecks that may exist in the process. While balancing budgetary concerns, training, and complexity of automation, laboratories should automate as much or as little of their existing processes [as] their comfort level with technology [allows]."

Shen's specific recommendations include heavy investment in automation such as parallel extraction/processing, liquid-handling robotics, online extraction/chromatography, chromatography multiplexing, and ultrahigh-pressure LC to improve turnaround and data quality.

The business case for automation is nearly identical for every workflow and boils down to greater consistency, fewer errors, and freeing up workers for other

"Most people don't appreciate how significant a problem sample prep poses for laboratories," notes Zoe Grosser, Ph.D., director of marketing at Horizon Technology (Salem, NH). "There's a lot of talk about the instrumentation and analytical methods. Sample prep is key to obtaining good results, yet it has been ignored over the years. Usually the most inexperienced people are assigned sample prep to learn their craft. It makes sense to improve an area where so much error occurs and to which so little attention has been paid."

INSIGHTS ON AUTOMATED SAMPLE PREPARATION

PREPARING EXACT CONCENTRATIONS IN JUST THE RIGHT QUANTITIES

Arguing against lab automation is becoming more difficult, particularly for sample prep. With reagents and solvents costing what they do, a business case based on direct cost savings for critical materials stands on its own.

Conventional sample preparation generates on a cost or weight basis much more waste than product. Mettler Toledo estimates that more than 99 percent of prepared solutions are never used and that manual prep of samples and standards for analytical methods accounts for up to 82 percent of a lab's solvent usage, 61 percent of labor time, and 49 percent of out-of-specification (OOS) errors. Moreover, continuing improvements in data management capabilities—the second-leading time killer in a lab, at 27 percent—will have the effect of increasing the share for sample preparation in nonautomated laboratories.

"Creating standards 'on demand' in appropriate volumes can cut solvent use by 95 percent."

"Manual handling always entails the risk of process failure and unacceptable variability from user to user," says Dr. Carsten Buhlmann, international product manager for automation at Eppendorf (Hamburg, Germany).

The most obvious effects of OOS errors are rework and time wasted in the (often vain) attempt to identify, remediate, and report on the root cause of the error. Rework, arguably a lab manager's worst nightmare, involves redeploying personnel, instrumentation, reagents, etc., to a job that should have been completed the first time and diverting those resources from new work. According to Mettler Toledo, OOS results cost between $3,000 and $10,000 and can shut down critical lab functions for anywhere from three days to several weeks, resulting in serious loss of revenue and/or productivity.

Laboratories with the capability of taking a lean/six sigma approach to OOSs go through the normal drill of analyzing and identifying waste and variability. This approach quickly leads to the observation that the more human steps involved in a process, the more likely the incursion of systematic and nonsystematic errors.

According to Dr. Charles Ray, former associate director of analytical R&D at Bristol-Myers Squibb and currently a consultant at CWR Consulting, a formal lean program is often not necessary to resolve OOS incidents. "A team can simply sit down and go through a very detailed workflow chart and highlight the problem areas. These may be steps that no longer make sense, that have a high likelihood of involving both determinate and indeterminate errors, and where efficiencies and cost reductions can occur."

Gravimetric (weighing) automation is becoming an acceptable strategy for avoiding overprep or inaccurately formulated solutions, yet most systems remain unautomated. Automated balances still require operators to add and remove vials. Their main benefits, as noted by Joanne Ratcliff, Ph.D., an analytical chemist and current communications project manager at Mettler Toledo, involve recordkeeping and allowing investigators to prepare just the right quantities of buffers or reagent solutions.

A typical system, exemplified by Mettler's Quantos instrument, employs both solid and liquid dispensing to create solutions of exact concentration in any container. After the system dispenses the solid, it adds precisely enough liquid for the desired concentration. The two-step process eliminates the burden on the technician of weighing solids and dispensing liquids precisely. When the operation is complete, Quantos prints a label describing exactly what is in the vial and records that information.

"The benefits are that you can now make up much smaller volumes of sample," Dr. Ratcliff tells *Lab Manager Magazine*. "That's because the minimum weight is lower than what might be accurately measured by hand, and you don't need to round up the liquid levels in a volumetric flask." For example, a worker expecting to make 50 HPLC injections of 10 microliters each can accurately produce one mL of solution instead of having to fill a 10 mL or 100 mL volumetric flask. This approach also shields workers from potentially toxic solids and replaces expensive volumetric glassware, which requires cleaning, with disposable, low-cost vials.

DOLLARS AND CENTS

The cost of automation is always a consideration in lab managers' decisions on whether to automate. "Some decision makers think they will save money by not automating," says Mehul Vora, global product manager in Beckman Coulter Life Sciences' (Brea, CA) automation group. "But in the long run, as automated sample prep becomes a routine part of your lab, the quality improvements and wiser deployment of human resources will pay for themselves in a very short time."

▲ *Liquid Handling System / Encore Multispan / Agilent Technologies / www.agilent.com*

Real life experience confirms this view. Analysis by Agilent indicates a rapid return on investment for sample and standard prep automation based on the cost of labor, glassware, lower consumption of reagents and solvents, and reduction of rework.

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A technician spending just five minutes on each of 300 samples per week costs the lab about $7,000 per month, assuming salary and overhead at $70 per hour. Creating standards "on demand" in appropriate volumes can cut solvent use by 95 percent and reference standard consumption by up to 75 percent. An Agilent customer reported solvent-related savings of $16,000 per year for just one assay, including much lower disposal costs and savings on glassware replacement and cleaning of more than $60,000 per year.

A case study from Horizon Technology summarized in Table 1 illustrates direct cost savings plus labor savings from switching from manual to automated prep.

BENEFIT	COST SAVINGS	COMMENTS
Reduced solvent use	$9,336	Reduction in solvent use
Reduced waste disposal	$3,960	Less waste generated
Reduced labor	$16,688	Reduction of overtime, greater flexibility
Reduced solvent contact	unknown	Safety
Reduced glassware use	$15,055	Minimized replacement costs
TOTAL	$45,039	

The $45,039 in savings represents an approximately three-year payback on a $141,000 system consisting of an eight-position extraction system, a DryVap add-on for drying and concentrating samples, and vacuum pumps. Direct ROI was only a minor part of the equation for this customer, however. They estimated that the automation capability enabled them to take on approximately $150,000 per year in more-complex contract work in addition to retaining Method 525.2 in-house, valued at $117,000 yearly. EPA Method 525.2 is a complex drinking water analysis protocol involving a very large list of analytes. Solid phase extraction enabled this lab to meet the method's strict quality requirements more easily.

All the arguments for automating sample preparation are equally valid for standards prep. Standards preparation rivals sample preparation in repetitiveness, reliance on precise measurement, and in many cases, the number of units. While standards prep is somewhat more predictable in terms of operations (dispensing, diluting, etc.), it is no less critical to data quality. Some protocols, particularly in food safety testing, call for preparing dozens of potential reference analytes at several concentrations each.

Automating standards preparation saves reagents and samples by producing the right quantity of standard solutions. As with sample prep, technicians tend to "overdo" standards prep, sometimes creating 10 or 20 times as much stock solution as may be needed. Automated systems can be set up to make up just enough solution for one day's assays fresh at the beginning of the first shift. "Labs are much less likely to use expired standards when they have an automated solution to do all the work," observes Agilent's Peter Mrozinski.

As with sample prep, automating benefits standards prep not so much through speed as by introducing consistency and efficiently utilizing human resources. Raw sample numbers play a surprisingly insignificant role in the decision to automate.

"Automation's contributions are its consistency and reproducibility," Mrozinski adds. "It's as accurate as your best technician on a good day but with the reliability and reproducibility one expects from an analytical instrument."

In November 2012, Agilent introduced the Encore Multispan Liquid Handling System for advanced automated sample preparation. Encore combines innovative pipetting with a built-in robotic arm that automates a substantially larger portion of sample prep workflows while increasing walkaway time.

Equally important for a lab's business and worker satisfaction, automation creates an atmosphere of accomplishment. "In the end, a robot isn't necessarily any faster than a skilled technician," says Jason Greene, product manager at BioTek Instruments (Winooski, VT). "But it does provide uniformity and performance, accuracy, and precision, and it gives workers the opportunity to be elsewhere."

◀ *Deep Well Washer / ELx405™ Select / BioTek / www.biotek.com*

INSIGHTS ON AUTOMATED SAMPLE PREPARATION

LIMITATIONS OF SAMPLE PREP SYSTEMS VARY BY INSTRUMENT TYPE AND SAMPLE

▲ *Liquid Handling Workstation / Biomek 4000 / Beckman Coulter / www.beckmancoulter.com*

Despite protestations to the contrary, automation is not something to undertake casually. Due to the dizzying array of possibilities and workflows, the technology has not yet achieved the simplicity of consumer products.

Given the diversity of analytical samples, the first few steps of sample preparation have little in common. Consider how one might acquire and initially process an orange, oil sludge, soil, or water. The first few steps might consist of filtering, grinding, adding stabilizers, sonicating, or a hundred other things. Convergence in prep operations and techniques occurs as the sample gets closer to the actual analysis. At this stage, diluting, dispensing, reconstituting, adding reagents, chemical derivatization, and mechanical operations such as heating, cooling, mixing, and extracting become more standardized.

One area that remains challenging for automation is primary sample handling: acquiring samples and getting them into a suitable format for further study. A geologist or an environmental scientist must still travel to the field to collect rock or water samples and get them ready for lab work. Some primary steps, such as drawing blood or excising tumor tissue and rendering it into a plate or tube from which it is accessible to the automation system, involve a very high level of skill. "While these examples require a good deal of human intervention, there are opportunities to address those types of workflows as well," notes Jeremy Lambert, director, automation and liquid handling at PerkinElmer (Hopkinton, MA).

"Convergence in prep operations and techniques occurs as the sample gets closer to the actual analysis."

A typical sample workflow may involve collecting the sample, subjecting it to the reagents and purification, transferring it to some form of labware, applying physical/mechanical conditions (heating, cooling, shaking, amplifying), and introducing it into the analysis system. "A lab technician can perform all these operations by hand," notes Mehul Vora of Beckman Coulter, which just introduced the latest product on its Biomek platform, the Biomek 4000 Laboratory Automation Workstation. "But the scientist is after only the data." Nothing that occurs before that DNA analysis or spectroscopy reading adds value to the scientific exercise, Vora

explains. "If scientists could wave a magic wand and have the sample ready for analysis, they would do that. That's not the real world, but the relevant question is what is the best way to get there?"

> "If scientists could wave a magic wand and have the sample ready for analysis, they would do that."

PHYSICAL LIMITS

Limitations of sample prep systems vary by instrument type and sample. Most are related to the fact that samples tend to be fluids—but not all liquids are created equal. Viscous samples require more work, time, and care than standard samples do. "Many viscous samples are difficult to filter," observes BioTek product manager Jason Greene. "You may have to increase the vacuum, pull on the sample longer, or watch it more closely to get through that step."

For drug assays, the predominant solvent, dimethyl sulfoxide (DMSO), presents assaying problems due to its hygroscopicity. Solutions rapidly equilibrate in laboratory air to 70 percent DMSO and 30 percent water, which can interfere with many assays or provide erroneously low concentrations. Companies looking for very accurate inhibition constants for drug candidates need to take this into account.

From a concentration standpoint the inverse of hydration is evaporation, which is always an issue with very low-volume, water-based assays. Greene hears about this problem "a lot, which is why many customers have backed away from ultralow-volume 1536-well plates and are beginning to readopt 384- and 96-well formats."

Finally, Greene warns lab workers to know the limitations of everything they work with, including complex sample prep systems. "It's funny how diligent scientists can be in testing everything except instruments. They don't realize the wide variability that can occur over time. It takes way longer to troubleshoot bad results than to perform due diligence up front."

Vendors of prep equipment and systems, reagents, kits, and labware strive mightily to accommodate the need to automate routine lab tasks, but what are the limits? We know that an automated liquid handler cannot grind tissue or dispense many liters of solvent at once. Yet they have the bases covered for most preparative workflows in the life sciences.

"When we ask our experts, they say anything related to life science workflows is possible," notes Mehul Vora. "Give me the assay and I can automate it, provided it fits into a tube or a microtiter plate." What about an assay that does not fit the standard ANSI/SLAS (formerly ANSI/SBS) format or was previously not automated or is perhaps too complex and operator-centric for standard automation tools? With the input of assay vendors, engineers from Beckman's Integrated Solutions division can design labware that enables automation of many customer workflows.

LEARNING CURVE

For labs unfamiliar with automation, particularly for sample prep, vendors such as BioTek Instruments serve a vital role in dispelling doubts and opening up vistas. "We know a lot of super high-end systems are out there," Greene observes. "What we offer is that same general capability in a smaller, more affordable format. Like many automation firms, BioTek reaches out to equipment and reagent companies to combine their analytical capabilities with BioTek automation systems. Examples include an off-the-shelf vacuum manifold that required a special labware holder and an oligonucleotide sequencing reaction cleanup kit, which was automated on a BioTek platform. Both additions resulted from a collaboration with Millipore. BioTek's other automation partners reads like a Who's Who of laboratory equipment: Caliper, Hamilton, Agilent, Tecan, Beckman Coulter, and others. These efforts are "very well received" by reagent, kit, and labware companies as well as by customers, Greene says.

Despite operating at what one might call the entry level of automation in terms of throughput, BioTek is integrated within the automation market, selling detectors, robots, liquid handlers, and data systems. Of the latter, Greene believes that the software and

interface should match the ease of use of the automation system itself. "Users don't want to spend days writing a liquid handling protocol." High-end systems can offer significantly more capability but cannot always boast the same user-friendliness.

"Lab workers [need] to know the limitations of everything they work with, including complex sample prep systems."

CARE

Care and maintenance of automated sample preparation systems are difficult topics because so many types of "sample prep" exist. Limited to liquid handling systems as we are, one finds the usual issues of replacing valves and washers and maintaining cleanliness. Rinsing is critical after a run on a liquid handler. With pipetting tools, O-rings may require replacement every few months, depending on frequency of use and type of liquid. Simple tests determine whether a pipette seal has been compromised.

A common issue with automated microplate washers, for example, is buildup of proteins and salts, which clogs equipment. The most effective way to remove this buildup is through ultrasonic cleaning. The manifold is typically removed from the instrument and placed in an ultrasonic bath for cleaning. BioTek offers integrated ultrasonic cleaning so that the manifold can remain in place and the cleaning can be preprogrammed and run without user intervention.

Filtration System / Samplicity® / EMD Millipore / www.millipore.com

In the end, failure to understand what manufacturers recommend for routine care is one of the biggest maintenance issues, says Greene. "There are things with liquid handlers that you should be doing daily, weekly, monthly. Given the high turnover in some labs, this doesn't always happen. When things are let go for too long, you wind up with cumulative errors—you reach a point where your data is no longer within acceptable tolerances. Care and maintenance don't take a lot of time, but they need to be done based on the manufacturer's recommendations and the lab's workflow priorities."

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IMPROVED SAMPLE PREP SPEED AND ACCURACY DEPEND UPON WORKFLOW

A comprehensive review of sample preparation would require a multivolume work. Beyond that, covering the possible number of workflows given the hundreds of potential "unit operations" would fill an encyclopedia, especially when solids (rock, soil, animal tissue) are considered.

ENVIRONMENTAL

While the major market share for automated sample prep lies in the life sciences, which operate with very small samples, many advantages are achievable outside biology, where larger samples rule—albeit perhaps not always at the same level of automation. For example, Horizon Technologies specializes in automating the extraction of large volumes of aqueous samples, principally for environmental analysis. EPA methods call for collecting up to two liters of water when testing for various contaminants. Due to sometimes very low concentrations of analytes, some tests demand collection of even larger volumes.

Horizon's SPE-DEX 4790 automated extractor system accepts sample bottles without manipulation. Samples are initially filtered to remove particulates and then adsorbed onto a solid phase extraction disk. The system then extracts analytes from the disk. Additional concentration steps, which render the sample suitable for LC or GC analysis, are also automated. Up to eight extractors may be run simultaneously through a single computer controller.

"Most testing labs are still using 'bucket chemistry' to process large-volume samples."

➤ *Sample flow through automated extraction, drying and evaporation/concentration processes.*

"Most testing labs are still using 'bucket chemistry' to process large-volume samples," says Zoe Grosser. "Conventional extraction, drying, and concentration from volumes that large can take anywhere from one to three hours, depending on the sample's complexity and the analytes of interest. Here it takes just 30 minutes per extraction, and it's a walkaway operation."

Grosser outlines the usual list of benefits: increased user productivity, decreased solvent consumption, fewer errors, greater consistency, lower labor costs, fewer re-extractions, freeing workers for other tasks, reduced glassware purchase and cleaning (the extractor cleans sample bottles after emptying them), and improved external ROI. "Labs are able to convert more samples per unit time, reduce customer support costs, and improve brand value of their services by informing customers of their automation capabilities. Automation sells."

GENOMICS

In high-throughput genomics, assays are lengthy, consume many thousands of dollars in reagents, and are limited by tissue availability and quality. Sample preparation can consume several days. "Researchers don't know how the tests are going until the very end," says PerkinElmer's Jeremy Lambert. PerkinElmer's key market areas are high-throughput genomics (mostly next-generation sequencing assays) and biotherapeutics discovery.

Kits originally developed for high-throughput genomics were designed for manual lab processing: An operator with a multichannel pipette might process up to 15 samples at a time through a workflow several days long that is highly demanding of hands-on time. "Transforming bench-worthy workflows to automation while maintaining quality requires significant optimization," Lambert says.

> "In high-throughput genomics, assays are lengthy [and] consume many thousands of dollars in reagents."

Genomics labs tend to conduct similar protocols that rapidly evolve due to improvements in techniques, methods, reagents, and scientific understanding. Vendors introduce new protocols approximately every three to six months. To prevent "method creep," some vendors employ single, fixed configurations for much of their liquid handling equipment, so new methods can be deployed to the installed base very quickly.

PerkinElmer's other specialty, biotherapeutics, involves a completely different set of tools. Where genomics chews up DNA and RNA to determine their molecular sequences, cell-derived proteins are analyzed for size, purity, charge, activity, and other critical quality attributes, usually by high-performance liquid chromatography and LC-mass spectrometry. Sample preparation is critical to ensure that analytical tests provide the right information in a competitive environment where one-day delays in market entry are punished. "In protein labs researchers are highly trained biochemists who need to focus not on sample preparation but on more challenging tasks, such as interpreting assay results. Automation frees them to do so."

MICROFLUIDICS: HOW LOW CAN YOU GO?

DNA extraction from cell cultures for subsequent amplification is an example of a complex sample prep that is often the rate-limiting step of a microbiological workflow. DNA is separated on microscale chromatography columns in a process that requires pipetting, dispensing, and pressurizing to elute the sample through a Q-Sepharose column. All three functions are available in automated formats but typically as separate instruments. A group at the Fraunhofer Center for Manufacturing Innovation at Boston University (Brookline, MA) has developed a prototype device, the Tripette, which combines the three essential functions through a six-by-six-inch microchanneled polycarbonate manifold that sits above a standard microtiter plate. Backing up the Tripette are standard fluid delivery and pressure systems. "The innovation is not the services behind the scenes but the manifold itself," says Alexis Sauer-Budge, Ph.D., one of the Tripette's inventors. Fraunhofer is interested in

codevelopment and collaboration on the Tripette and other sample prep technologies found at the website http://bit.ly/RImeSs.

In contrast to the almost macroscale Tripette, Akonni Biosystems (Frederick, MD) has built an integrated, microfluidic-controlled microarray platform for sample prep and analysis of genetic mutations known as single-nucleotide polymorphisms (SNPs). Targeted at forensic applications, the microarray incorporates Akonni's gel drop microarray technology, a thermal cycler, a reader, and a cartridge dock in a single-use "sample to analysis" microchanneled platform.

"DNA extraction from cell cultures for subsequent amplification is an example of a complex sample prep."

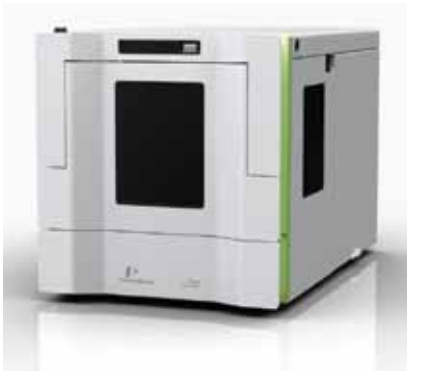
A WORD ON SPEED

A misconception regarding automation in general and automated sample preparation in particular is that it improves throughput. This line of reasoning suggests a barrier—some number of samples per day—before which automation is unwarranted and beyond which it becomes sensible.

According to Beckman's Mehul Vora, the "throughput" argument collapses in the face of the realities of quality and workflow. "Intuition says if you're dealing with just a few samples, you might just as well do them by hand because it's faster than setting up a machine. But our customers tell us otherwise." Automation's major advantages over manual sample prep are fourfold: avoiding human error, eliminating operator-related contamination, enhancing data quality, and maintaining consistent results.

Manual sample prep offers the illusion of control over the process. The question is what is controlling whom? A prep may take five steps that are mostly walkaway; for example, weighing, dissolving, filtering, adding reagent, and heating. Each operation may require only ten or 15 minutes of attention, but the process keeps drawing the scientist or technician back from other work. Five samples can easily keep a lab worker tied to prep work for half a day, even more if operations require monitoring. "And if the number is 55 samples, the value of automation becomes even more clear," Vora says.

Automation does not necessarily speed up prep work. If one includes time to set up the method, absolute throughput—the number of samples processed per day—may hardly be affected. If productivity is measured by what a typical worker can accomplish in a shift, however, the equation changes. Now, instead of babysitting samples and individual operations, a worker may spend half an hour setting up reagents and selecting a method, occasionally moving plates, taking a few peeks during the day. In other words, the worker is freed to perform less repetitive, more challenging tasks. "Even low-throughput labs can save value-added time."



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Automated Extraction System / SPE-DEX 4790 Horizon Technology / www.horizontechinc.com

INSIGHTS ON AUTOMATED SAMPLE PREPARATION

A Q&A WITH SELECT AUTOMATED SAMPLE PREP EXPERTS

OUR EXPERTS:

Gang Xue, Ph.D.,
Associate Research Fellow
Pfizer
Groton, CT

Dhara Patel
Research Instructor
Washington University
St. Louis, MO

Q: Describe your sample prep workflow(s).

A: **Gang Xue:** The typical samples involved in pharmaceutical analysis include raw materials, intermediates, active pharmaceutical ingredients, excipients, and formulated drug products. Reference standards are used for quantitative analyses. The majority of sample preparation methods target chromatographic potency and purity analyses, such as for developing processes and performing release or stability testing. We use the Mettler Toledo Quantos; FreeSlate Powderium; and tablet extraction systems such as Sotax TPW3, RTS SoliPrep, Polytron, and ASE (accelerated solvent extraction). Hamilton Microlab and CTC autosampler are also used for sample dilution.

Dhara Patel: We employ two types of automation. We use what I classify as full-automation for our compound screening, or HTS. This takes more effort to set up and validate the entire process, but minimal manual intervention is required once the process is validated. We use liquid handlers, plate handlers, automated incubators, and readout instruments like plate readers and automated imagers. The manual intervention required involves stacking plates and placing reagents when an operation starts. The other type of automation we perform is what I classify as semi-automated, which automates only the most labor-intensive and repetitive steps. We use semi-automation for validation assays after HTS and also for any basic science experiment. In this case, to decide whether a process is worth automating, we weigh the effort required to fully validate the steps against performing the steps by hand.

Most of our samples involve cell lines that are treated with cytokine and/or small molecules, staining of cells thereafter, readouts using plate readers, and an automated imager. We are now preparing to automate such processes for primary cells that require growth on air-liquid interface and work with live viruses (BSL2). This requires automation in a biosafety cabinet, which is the main challenge.

Q: What are the principal benefits to your organization of automating sample preparation?

A: **Gang Xue:** The key benefits include resource savings and enhanced method robustness. Sample preparation and data analysis are the most labor-intensive unit operations in pharmaceutical analyses. An automated system reduces the time for scientists to develop and operate the sample preparation method. A recent trade publication survey reported that sample preparation consumes about 60 percent of a scientist's time and that approximately 30 percent of out-of-specification (OOS) errors are due to sample preparation.

In a regulated GMP environment, investigations of OOS results generally take significant time and resources. Via the use of automation, we reduced the sample preparation time for a sustained release tablet from 24 hours through manual methods to 15 minutes. An added benefit was the significantly improved precision of the assay. In most cases, automation doesn't necessarily reduce the overall cycle time of the analysis because of the sequential nature of most automated systems.

Dhara Patel: The biggest advantage of our fully automated process for HTS, especially for a phenotypic cell-based screen, is data quality from consistent timing between plates. This and our data analysis technique enable us to compare data from multiple screens, which for an academic lab like ours that is not a core and can afford to screen smaller libraries at separate times, often months apart, is extremely important. In the long run, being able to hit-pick from multiple screens collectively saves a lot of effort invested in hit-validation and further follow-up. For our semi-automated processes, the biggest advantage or benefit is time savings. When validating any automation process, we always compare to data obtained by performing the experiment by hand. So we can quantify data quality.

Q: What sources of methods or techniques do you rely on most for automated sample preparation?

A: **Gang Xue:** Usually lab automation-related conferences and trade shows—such as the Pittsburg Conference, Lab Automation Conference (SLAS) in the United States, and Analytica in the EU—are the most valuable sources for innovative automated sample preparation techniques. The precision of the automated methods varies by the scale, throughput, and unit operation. However, the method robustness is mostly reported to be on par with or better than manual methods. The reliability of the instruments can be an issue though. Commercial instruments are typically more reliable than custom-built systems, and smaller systems targeting limited unit operations are better than large, integrated solutions. Additionally, these smaller, targeted systems are more cost-effective and typically more user-friendly, resulting in general lab use, not just use by specialists.

Dhara Patel: For broad-stroke methodology and learning about new methodology, I rely on publications in journals. When starting a new methodology, I look for any algorithms or workflows or examples that may have been developed by the automation equipment company. When troubleshooting, I turn to message boards online. I talk to tech experts from the companies. I also talk with sales representatives, especially to keep up with what's new in plasticware. When I read journals or magazines, I look for broad-stroke ideas. When I read information from vendors, I'm looking for details in methodology. Generally speaking, I think what I read is reliable. However, I always think that the devil is in the details and care needs to be taken when implementing what I read for my own use.

Q: How can vendors of automation equipment and reagents/disposables improve the quality and consistency of automated sample or standards prep?

A: **Gang Xue:** Building smaller modular systems that sell to relatively large user bases would help improve the overall robustness of the instruments as well as reduce operational complexity while lowering costs. Providing standardized hardware and software interfaces to allow easy integration (plug and play) would enable both workflow flexibility and reliability. Standardization of consumables would also be welcome. Many of the challenges with robotic operations are related to the different sizes and shapes of the vessels they have to manage.

Dhara Patel: In general, I think that vendors of both automation equipment and reagents and disposables are good at listening to understand what the customer requires. But I think more progress would be made if more attention were given to smaller markets of niche applications. When purchasing equipment, I think what needs to happen more is that the vendor needs to understand what the usage is going to be and discuss equipment options. Many times details of one system (or even a sample preparation platform) are discussed without paying due attention to whether it would be the best choice for the application.