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INSIGHTS
LAB TECHNOLOGY BUYER'S REPORT

INSIGHTS ON AUTOMATED LIQUID HANDLING

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CONTENTS

INSIGHTS ON AUTOMATED LIQUID HANDLING

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IT'S NOT ONLY ABOUT THROUGHPUT

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Automated liquid handling (ALH) grew from the high-throughput needs of medical diagnostic labs in the late 1980s through the 1990s and then received an additional boost from genomic sequencing. As it evolved from a specialized, expert-driven instrument system to a generalized laboratory workstation, ALH has become more accessible to more workers in more labs.

Automation vendors have achieved this despite the uniqueness of ALH workflows. ALH is not a point and shoot technique like spectroscopy or analytical chromatography. Every ALH experiment is unique in terms of reagents, volumes, order of addition, target analyte, and readout.

Speed is the most-cited advantage of ALH compared with manual pipetting, but as we will see, pipetting accuracy and freeing lab workers from monotonous pipetting are valued at least as highly as throughput. Depending on the number of samples a lab typically runs, the business case for acquiring or upgrading an ALH system may be based on throughput, quality, operator time savings, or some combination of the these factors.

Accuracy provides an additional, independent cost-saving benefit. ALH can ensure the accuracy of ultra-small volume dispensing, which is a principal factor in minimizing assay, reagent, and disposal costs. Microliter-size assays are now routine, with PCR often employing nanoliter or picoliter reaction volumes and microarraying striving for femtoliter dispensing. Reduced volumes increase an assay's volumetric complexity, which in turn raises the standard for accurate pipetting.

Assay miniaturization has spilled over into instruments and components as well. "Lab space is limited, and the less space taken up by instrumentation, the better," says Merja Mehto, product manager for automated liquid handling products at Thermo Fisher Scientific (Vantaa, Finland). This has led vendors to devote significant design resources to creating liquid handlers that are scalable, compact, and easy to use.

Given that every liquid handling workflow is distinctive, the greatest challenge facing the ALH industry is providing "average" laboratories with customization at a reasonable cost. (As one unnamed vendor confided, "Customers want everything.") Vendors achieve customization through standardization of consumables and reagents and in investments in instrument interoperability.

OEMs also provide validation packages for instruments and reagent kits, greatly reducing this tedious process in users' workflows, notes Sikander Gill, Ph.D., of Aurora Biomed (Vancouver, BC). "This enables customers to purchase both the application and the solution, while vendors benefit from continued sales of consumables." The evolved product mix may result, he says, from "OEM partnerships" or through diversification of research and development teams within the equipment company.

SOFTWARE

Some experts would argue that software and interfaces have been the most significant areas of change for scientific instrumentation. "ALH software needs to keep pace with customer needs," says Gill, by providing ready access to "complex assay and integration" capabilities through a user-friendly interface.

Advances in computing and data storage began in earnest with Microsoft DOS. Microsoft Windows® provided a glimpse into the possibilities of graphical user interfaces (GUIs), particularly for "distributed" workstations such as liquid handlers that may be connected to several other instruments. But even with graphical interfaces, users faced system-level involvement in programming and engineering. "We were conditioned to accept a situation that wasn't quite perfect," says Tom Osborne, product manager at PerkinElmer (Waltham, MA).

▼ *Automated Homogenizer Liquid Handling System | LH96*
Omni International | www.omni-inc.com

Thought leaders at research centers still interact with instrumentation in this manner, but the underlying philosophy of instrument control is changing. This isn't because instrument experts are disappearing from universities (even as their numbers dwindle at companies); it's due to a shift in user expectations: end users and their employers increasingly stress walk-up operation and short learning curves, and vendors comply.

> "ALH can ensure the accuracy of ultra-small volume dispensing."

Younger scientists raised on electronic gadgets, portability, interconnectivity, and out-of-the-box usability are pushing the boundaries of software and interface expectations even further. "Vendors need to invest in GUIs that are more 'purpose built' for walk-up utility for mainstream audiences who are interested in answering scientific questions in 12 minutes, not in method development or engineering," Osborne comments. "At this stage all the major vendors are passionate about this."

According to Greg Robinson, director of automation products at Gilson (Middleton, WI), vendors dedicate significant effort to software and interfaces, specifically so that:

- Overall robustness prevents crashes.
- Drag-and-drop functionality creates methods more rapidly and reliably.
- Interactive, dynamic task pages update automatically based on the user's selection; for example, hiding or uncovering certain fields in context, depending on the selection. Pages should display only the required entry boxes and selections. This improves software usability, reduces mistakes, and ultimately increases the efficiency of method development.
- The ability to automatically optimize the application or protocol effectively reduces instrument wait time.
- They allow interaction with peripheral devices such as balances, bar code readers, chillers, heaters, pH meters, shakers, and anything else that affects the workflow.
- Manual controls (related to simulation) allow users to perform base-level functions such as priming solvent lines and troubleshooting error conditions or application issues.
- Simulation. Users require modeling capabilities to test methods visually on a computer screen before actually running the instrument and wasting reagents and precious samples, etc. This advanced feature allows users to spot any issues that may negatively affect either the experiment or the instrument.

"Users want a system where they can push a button and leave," says Tom Osborne, "but one that is flexible enough to incorporate changes in method or workflow." This level of trust in a complex instrument with many moving parts is difficult to achieve without some sort of simulation function.

"The greatest challenge facing the ALH industry is providing 'average' laboratories with customization at a reasonable cost."

CELL-BASED ASSAYS

Expanding beyond traditional reagent- and solvent-dispensing markets is a sign that ALH is maturing as an industry. Cell-based assays, a high-growth area for microtiter plates and by extension ALH systems, are an exciting area for innovative liquid handling on a small scale. Cell-based assays are used to screen drugs, cosmetics, and chemicals of environmental concern, as well as to test cells themselves for viability, productivity, or bioremediation worthiness. As with other types of assays, test volumes are reaching microscopic proportions, with many vendors selling high-density (up to 1,536-well) plates with microliter working volumes suitable for cell work. BioFluidix claims single-cell delivery capability, for example, for its PipeJet.

Automation provides consistency for cell-based assays, but conventional wells do not reproduce physiologic conditions very well because the cultures are two-dimensional. Cells in nature exist in a complex three-dimensional matrix, not in solution. At the 3D Cell Culture conference (part of the huge Dechema exhibition series) held in February 2012, 3D Biomatrix (Ann Arbor, MI) introduced "Hanging Drop" cell culture plates in which cell volumes are maintained as suspended droplets accessible from either the top or bottom by conventional liquid handling equipment. Shortly after depositing the droplets, cells form physiologically relevant "spheroids," which may be tested by drugs or reagents.

Speed is also an issue with cell-based assays. Completing the protocol before resident cells have the opportunity to change through growth, senescence, or death is critical for obtaining consistent results. Another source of inconsistency with living systems is degradation of assay reagents such as proteins, enzymes, or genes. Technicians tend to overuse prepared samples and reagents when time runs short. Speeding up the process reduces this tendency.

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HOW TO KEEP YOUR ALH RUNNING SMOOTHLY

Service and maintenance for liquid handling equipment are approximately the same as for a liquid chromatography system, but the type of service differs. All vendors offer typical levels of on-site service, with premium plans including preventive maintenance visits.

Post-sale support should be a top consideration for prospective buyers, says Sikander Gill. "Purchasing or selling an ALH system is easy, but maintenance requirements may overwhelm the average user. Post-sale service agreements are critical for achieving the long-term benefits of automation."

▲ *Span-8 Pipette Tips*
Biomek P1000 | Beckman Coulter
www.beckmancoulter.com

Most manufacturers offer training at either the customer's or the manufacturer's location, but the trend is toward a quick start and a shallow learning curve. According to Merja Mehto of Thermo Fisher Scientific, users need to get right to work "without having to spend hours and hours in training sessions." The length and depth of training may vary considerably depending on the instrument and workflows. "It can be just hours for the simple instruments with straightforward software or up to several days for more complicated systems."

In the past, many companies maintained an official or unofficial automation department that supported automated liquid handling. The need for specialists has diminished as instrumentation became more reliable and robust, and software/interface took over many of the more difficult method and protocol tasks. Vendors have further responded to the "disappearing expert" through software and hardware that are more application-purposed.

The industrialization of ALH systems has not completely eliminated the industrial specialist-engineer, as similar advances have in spectroscopy or chromatography labs. Although specialists are dwindling in number, many companies still rely on them due to the nature of automation (lots of moving parts, instrument interconnectivity). Complex systems involving a liquid handling component are only as trustworthy as their weakest link. But paradoxically, more of the maintenance and troubleshooting burden has shifted onto end users.

Pipette tip calibration is even more critical for multichannel pipettors in high-throughput labs. Even relatively minor errors propagate quickly at 100 plates per day. Calibration is normally part of routine on-site service. Pipetting heads found to be out of specification are swapped for refurbished heads on the spot.

In addition to equipment vendors, numerous third-party service firms provide routine maintenance for ALH systems. Some, like Artel (Westbrook, ME), sell do-it-yourself service kits for validating the precision and accuracy of multipipette ALH systems. Given the criticality of consistency and accuracy, such kits are the way to go between service calls.

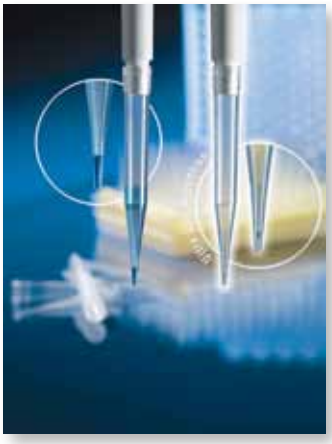
The calibration process for pipetting heads involves some high-throughput ideas. Artel's package includes software that runs on a desktop or laptop computer, sample solutions, optical-quality verification plates (96- and 384-well formats), calibration plate, plate shaker, either of two plate readers that quantify delivered volumes at two wavelengths, and a bar code reader.

ALH systems are not black boxes like mass detectors or nuclear magnetic resonance spectrometers. Laboratories committed to automation are generally not fearful of digging into the works, says Tom Osborne, especially for mechanical parts that wear out. Another common maintenance task involves "framing" the deck every few months to ensure that pipette tips dispense where they're supposed to. "Users can order the parts and repair the instruments themselves in some cases. More complex repair and maintenance are covered under the service contract that usually includes operational qualification—documenting that the instrument is performing within specifications."

Labs comfortable with automation become resigned to cost of ownership, training, and frequent ordering of consumables. These factors, according to Osborne, need to be managed and factored into the ongoing costs of doing business.

ALH systems are constantly moving, sometimes 24 hours a day, seven days a week. Motion involves friction and strains that are not evident in spectrophotometers, for example. ALH operators should be prepared to change belts and check pipette stage alignments to ensure that the robotics are not drifting. Smaller-format testing sets (e.g., 1,584-well plates) demand a higher level of mechanical precision than 1mL glass tubes do. And despite constantly upgraded hardware and vendors' dedication to interoperability, the interplay between ALH and third-party instruments is a source of error and inconsistency. "The best time to consider upkeep and preventive maintenance as part of cost of ownership is while you're considering the acquisition of automation." This is when potential customers should have a frank discussion with vendors about downtime, Osborne says.

One way to overcome downtime resulting from instrument glitches is to build redundancy into analytical operations. This is most often seen in chromatography labs, where methods reside on all instruments or on a central server, and components may be swapped among instruments. Obviously not every laboratory can do this, even for relatively low-priced instruments, and only well-heeled industrial labs would even consider it for ALH systems. "Most labs can't afford redundancy," Osborne observes, "but if the value of the automated application is high enough, then this strategy is something to consider."



▲ *Pipette Tips – BRAND BrandTech Scientific | www.brandtech.com*

ALTERNATIVES TO FULL-SCALE AUTOMATION

Throughput does in fact play a determining role in whether to acquire or upgrade automation equipment. At several hundred plates per day the numbers essentially decide; at the low end, particularly for tasks such as dissolution, serial dilution, plate replication or reformatting, or incremental dispensing to multiple plates, semi automated, parallel dispensing systems might provide greater value than a full-blown ALH can.

Known for its multichannel manual pipettes, Integra Biosciences (Hudson, NH) has recently introduced a product, ViaFlow 96, that is mostly manual in critical operations of pipetting and plate handling. The 96-channel dispensing head is controlled by push-button actuation, and pipette tip positions are stored on the instrument for both dispensing and withdrawal, as with fully automated systems. Because dispensing is controlled electronically, pipetting times are brisk—about eight seconds for 96 wells.

"An instrument like this allows technicians to handle up to 50 plates as easily as with a robot," says Alexander Studer, Ph.D., of Integra Biosciences. The ViaFlow 96 addresses simplicity, ease of use, and learning curve but not, unfortunately, versatility. "If you're thinking of picking eight samples from a 96-well plate, this instrument is not for you," Dr. Studer admits.

One would expect the "sweet spot" market for such an instrument to be cash-strapped, low-throughput labs that cannot afford full automation. While that demographic is certainly part of the market, in fact high- and low-automation end-user groups overlap significantly. Almost every lab at times operates in low-throughput mode, requires one-off plate prep for a few dozen plates, or has all available automated systems occupied. "A lot of people who have all the bells and whistles sometimes want a

backup or the ability to run several plates, but setting up the full-featured instrument is too much trouble," Studer says.

A step up from Integra's approach are "personal" ALH systems that satisfy what Gilson's Greg Robinson calls a "personal solution-based model."

Desktop ALH systems fill a niche market segment between manual pipetting and full-throttle automation for low- to medium-throughput applications. Tecan and Agilent, both of high-throughput robotics fame, and Qiagen also sell "personal" liquid handlers, as do Eppendorf (Hauppauge, NY), Biohit, and Dornier-LTF.

Biohit (Helsinki, Finland; now part of Sartorius) touts the world's most compact ALH, the desktop-sized Roboline™, which uses a single pipettor to deliver reagents to microtiter plates (up to 96-well) or tubes under computer control.

Roboline represents a natural progression from the hand-operated pipettes and electronic models that put Biohit on the map. According to the company, electronic pipettes comprise just six percent of the manual pipette market, but demand for them is growing twice as fast as for hand-operated models (ten percent per year vs. five percent). Roboline is fully automated, but unlike full-featured ALH systems, Biohit claims full walk-up usability by novice technicians.

▼ *Automated Liquid Handling Personal Workstation*
Roboline | Biohit | www.biohit.com

Similarly Dornier-LTF (Lindau, Germany) has introduced a "personal pipetting robot," the PIROs, which like Roboline boasts a desktop-size footprint and capability for biosafety cabinet operation.

Jesse Cassidy, automation product manager at Eppendorf, says personal ALH customers would not typically buy a fully automated system, and vice versa, but some crossover exists. "It depends on what they're using it for." Some large labs will use Eppendorf's epMotion® system for low-criticality operations like sample aliquoting or to get their feet wet with automation. The drawback of these instruments, however, is that anything they can do, large systems can do better.

Eppendorf's entry-level benchtop ALH has basic features similar to those of its competitors' instruments. But the company also sells higher-end models with more deck positions, plate grippers, mixing, heating, vacuum, and variable volume capabilities.

➤ *Automated Aliquoting Liquid Handler | CV2000*
Thermo Fisher Scientific | www.thermofisher.com

GOING AUTOMATED MEANS MORE CONSISTENCY AND FREEDOM

For companies processing hundreds or thousands of plates per day, one could build a business case for switching to an automated pipetting system on throughput alone. Robots are faster and less expensive to feed and care for than humans are. These kinds of highly parallel, high-throughput workflows are relatively rare, however.

For medium-size, smaller-throughput labs, the speed advantages of automation may be difficult to achieve. Keith Roby, global life sciences marketing manager at Beckman Coulter (Indianapolis, IN), puts it simply: "You don't always get a higher throughput when you automate. That point gets lost on a lot of people."

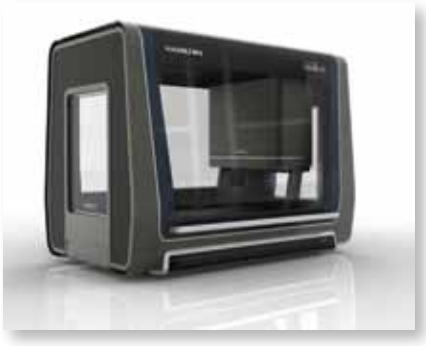
Today even small labs can achieve a solid return on investment from ALH systems. Automation enables scientists and technicians to work on more critical tasks while eliminating inconsistencies resulting from human error.

Inconsistency arises from pipetting errors that include incorrect volume delivery, mistaken reagent bottles, lost sample tubes, and variability due to technique. Humans also tend to overuse pipette tips, which can result in cross-contamination. The volumetric dynamic ranges involved in modern biological assays can also flummox human operators: no technician's eyesight is sufficiently acute to determine the dispensing accuracy of a two-microliter aliquot.

"Lab workers, in the U.S. and Europe in particular, are highly paid," adds Tom Osborne. In many situations a trained technician can execute a liquid handling method faster than a robot, especially when method development and instrument setup are taken into account. "But with automated systems these workers have the ability to walk away and do something more productive with their time." That, says Mr. Osborne, is a particularly attractive "avenue to return on investment. The more highly trained the technician, the greater the ROI. The monotonous nature of this sort of work is reason alone to want to walk away." Another factor is eliminating the risk of repetitive strain injury, which can incapacitate the brawniest lab tech. Manual pipette manufacturers learned long ago to devote significant design resources to ergonomics.

On the issue of time savings and consistency, automation provides another benefit: the ability to standardize and automate reagent and standards preparation, an extremely tedious and time-consuming process. When an experiment runs over several days, technicians are often tempted to refrigerate buffers and standard solutions and use them the next day rather than taking the time to produce them immediately before use. As Peter Mrozinski, product manager for workflow automation at Agilent Technologies (Wilmington, DE), points out, the value of ALH is most evident for standards preparation. One Agilent customer reported saving more than $35,000 in solvent costs

and rework in one year by employing a liquid handler to make up solutions and standards.



▲*Enclosed Benchtop Workstation | MICROLAB NIMBUS Hamilton Robotics | www.hamiltoncompany.com*

Sample preparation is a bottleneck in many workflows such as nucleic acid and protein extraction, PCR, and high-throughput HPLC analysis. ALH speeds up the process but does not address all sample-related issues. A sample prep employing magnetic particle technology is greatly accelerated when all reagents, beads, and even samples are quickly and reproducibly pipetted into plates before beginning a run. Many instruments combine sample prep and liquid handling functions. "However," Merja Mehto adds, "standard preps will still require extra centrifugation or a vacuum step and won't be accelerated significantly by ALH." Several companies—for example, Agilent—have ALH systems or workflows dedicated to sample and standards prep.

"You don't always get a higher throughput when you automate."

ALH has given rise to businesses based entirely on high-throughput dispensing. In February, BioStorage Technologies (Indianapolis, IN), which specializes in sample management for the life sciences, expanded its European operations to include sample preparation services. Services include sample aliquoting, nucleic acid extracting and verifying, and processing of blood cells (mostly for medical and pharmaceutical testing). The company also provides back-end tracking and inventory management for managing large numbers of samples. Again, the theme here is consistency and quality, which are to automation in liquid handling what ALH is to manual pipetting. Russ Hager, senior director at the company, described these services as "renewable resources" with complete audit trails for drug discovery. BioStorage counts among its customers the world's largest biopharmaceutical companies and clinical research organizations.

When discussing the acquisition or upgrade of an ALH system, vendors need to gauge the configuration the potential customer really needs. Sikander Gill compiled a checklist to ensure that customers receive the right amount of automation for their needs, with some room to grow:

- Throughput: The level of automation depends on the anticipated number of plates likely to be processed in a day, a week, or a year and how much downtime is expected. Liquid handling modules are commonly available in single-channel, eight-channel, or 96-channel formats. Higher sample numbers warrant systems with larger liquid handling modules and deck capacity.
- Quality and consistency: These cost more regardless of the instrument or system, although all significant vendors have by now achieved satisfactory levels for both.
- Workflows: Nucleic acid preparations, PCR, Sanger sequencing, next-gene library prep, ELISA, LLE, or SPE all have slightly different deck requirements. Some users prefer to carry out some operations manually, and some cannot afford end-to-end automation.
- Volume range: How much liquid does your typical experiment deliver? With what levels of precision and accuracy? These factors greatly affect the liquid handling module design and deck size requirements. Some workflows, for example, require multiple syringes and/or liquid handling arms.

▲*Laboratory Automation Workstation | Biomek FX^P Beckman Coulter | www.beckmancoulter.com*

A Q&A WITH SELECT AUTOMATED LIQUID HANDLING EXPERT END-USERS

OUR EXPERTS:

Alex Michel Ph.D., *Principal Research Associate,* Genzyme Corp., Waltham, MA

Luigi Francesco Covelli, *Microbiologist,* Medimmune, Mountain View, CA

Hugo M. Oliveira Ph.D., *University of Porto, Porto, Portugal*

Hardik B. Shah, *Contract Chemist,* Procter & Gamble, Mason, OH

Alex Nisthal Ph.D., *Postdoctoral Fellow,* Genzyme Corp., California Institute of Technology, Pasadena, CA

Q: What types of automated liquid handling instrumentation do you use?

A: **Alex Michel:** We use a variety of automated liquid handling systems in our lab. Primarily we use Tecan EVO 200s for the majority of our workload, but we utilize Tomtec and PerkinElmer ALHs in our weekly routine as well. Almost all of the systems are "stand alone," and the assay plates we generate from those systems are almost all used in some type of LC-MS analysis.

Luigi Covelli: The majority of our liquid handling [instruments] come from the Hamilton Company. We employ Hamilton STAR robotics to run the assay setup for our process and then batch-process our assays onto smaller, point-of-use liquid handlers. These instruments include Thermo Fisher Scientific's Multidrop with RapidStack plate handlers, and MDS Aquamax 2000/4000 plate washers with Stackmax plate handlers. For PCR-type applications, we use liquid handling robotics from Qiagen, specifically the QIAsymphony, M-48, Corbett CAS-1200, and QIAgility.

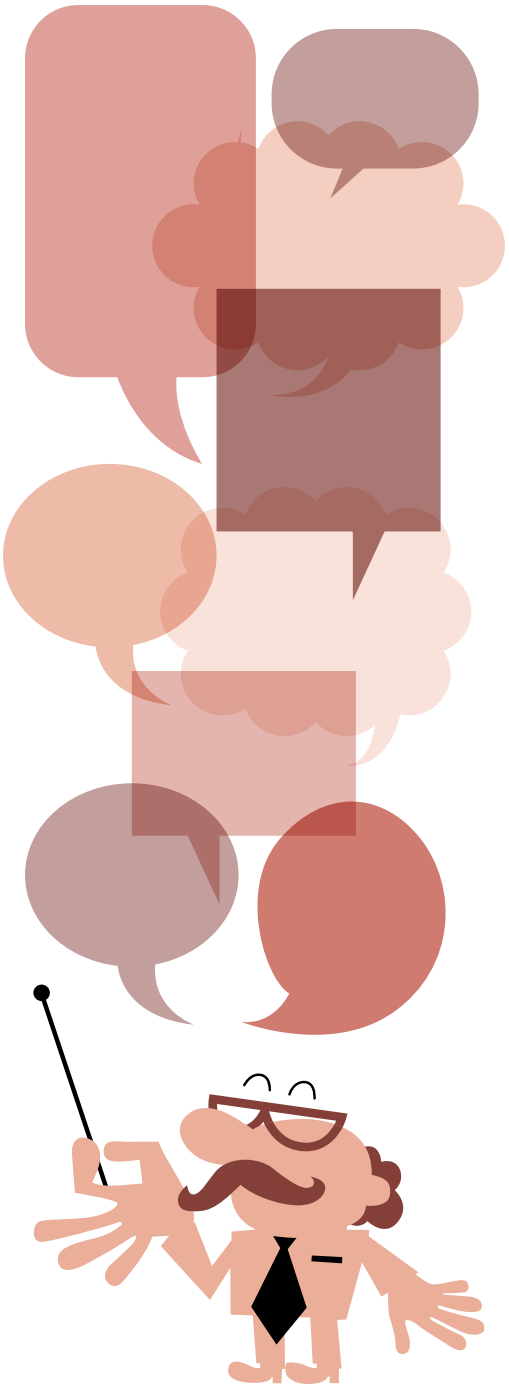
Hugo Oliveira: I'm a user of flow injection analysis and related techniques. In my case, the main goal of ALH is to ensure automatic and miniaturized sample preparation (analyte enrichment by solid phase extraction, or SPE) for liquid chromatographic analysis. The flow analysis manifold is coupled with the LC instrument for an automatic and integrated analytical protocol.

Hardik Shah: I work with the Rainin Liquidator and the Janus Mini-PROBE. We use the latter to transfer liquids from Bio Plas tubes [BIO PLAS, Inc.] to 96-well mass plates, typically at volumes from 20 to 1,000 microliters. The liquid handlers are not connected to any other instruments.

Alex Nisthal: We exclusively use a Tecan Freedom EVO system that has a lot of integrated bells and whistles, like a PCR machine, shaker, microplate reader, and vacuum unit. The Freedom EVO is contained within a biosafety enclosure, with waste lines and data cables leading out of the hood.

Q: Describe your "typical" workflows, and types of samples and experiments you run.

A: **Alex Michel:** Working in a DMPK department, our typical workflow centers around chemical property analysis, in vitro ADME studies, and sample processing for bioanalysis. We typically will use the systems to process samples for purity analysis, solubility assessment, and LogD determination. Acceptable compounds are then assayed for microsomal metabolic stability liabilities, CYP inhibition, and cell-based permeability, all of which are automated on our Tecan systems. Further down the line, we'll also handle samples for bioanalysis, plasma-protein binding determination, efflux potential, hepatocyte metabolic stability, CYP induction, etc. Again, all of those assays are either fully automated on our ALHs, or at least partially automated on those same systems.



Luigi Covelli: Our company tests samples from clinical trials, including whole blood, serum, plasma, nasal wash, and cell culture supernatant. We run a range of assays on these samples, for example hemagglutination inhibition, neuraminidase inhibition, microneutralization, viral genotyping and subtyping, fluorescent focus potency, $TCID_{50}$, and sample preparation. Typical automated assay workflows include sample ID verification, sample addition, serial dilution, transfer, and plate washing. Our use of automation is similar to a production line. Instead of automating the assay from start to finish, we use batch processing where specific steps are automated. With the implementation of our batch process automation, we've seen our throughput increase tenfold, and find this method suitable for our purposes.

Hugo Oliveira: My typical application comprises automatic SPE at microscale—either permanent or renewable sorbent approaches are possible—for analyte enrichment and matrix removal prior to LC injection. My work has been devoted to method development that comprised applications in the analysis of environmental samples, mostly water, and foodstuffs.

Hardik Shah: Approximately 90 percent of the time we use ALH to transfer liquid to 96-well plates for mass spectrometric biomarker assays. We run between three and four hundred such samples per day. Sample matrices typically consist of both aqueous and organic solvents, but occasionally human plasma. After transferring aliquots of samples to the 96-well plate, the samples are run on LC-MS-MS. The standards and QC results are quite consistent within and between batches.

Alex Nisthal: Typical workflows are operations that support our protein-engineering lab and another structural biology lab on campus. These include parallel site-directed mutagenesis, gene assembly, and protein purification. Assays include those measuring protein stability, HIV neutralization, and ELISA.

"Personally, I'd like to see more and better third-party support from the [ALH] vendors."

Q: What factors should purchasers of ALH instrumentation consider?

A: **Alex Michel:** Reliability, flexibility, ease of use, and redundancy all factored into our decisions. We wanted systems that we could count on, and that would also allow us to add onto them or reassign them with changing needs in the department. We really didn't want to be stuck with systems that could only run a single assay, so having multiple/flexible systems was very important to us.

Luigi Covelli: Vendor service and support are very important to us. As far as the instrument itself, purchasers should look for robustness, expandability to accommodate future needs, integration to other systems and operations, and programmability. And, of course, cost.

Hugo Oliveira: In my opinion, the main factors to consider before buying an ALH instrument are its robustness, including materials of construction, and its versatility for accommodating different analytical approaches. Software should also be "global," meaning it should control more than just the liquid handling system; [it] should perform data processing as well.

Hardik Shah: I have never been involved in the purchase of an ALH system, but I suppose throughput and reliability would be assets.

Alex Nisthal: When we purchased the EVO we were looking for a system that was extendable if our needs changed, and a brand that was highly reputable for precision. We also considered cost and ease of use.

Q: What can automation vendors do to improve their instrumentation, software, interface, or the general user experience?

A: **Alex Michel:** Personally I'd like to see more and better third-party support from the vendors. We typically go through the vendor of the system for additions to our platforms, but sometimes they don't have the exact tools that we're looking for and it can be difficult to implement a third-party solution. Having more options and easier solutions would give us more flexibility with how we use our automation.

Luigi Covelli: Increasing the pipette capacity volume has been an issue for the majority of the ALH instruments on the market. Only a few vendors have excelled on this factor, as well as on liquid level detection. Software and interface in general [have] a steep learning curve regardless of new software upgrades and advancements. Providing additional support for the life of the ALH would increase interest and help overcome the initial "scare" of users stepping into automated robotics.

Hugo Oliveira: In the case of flow analysis, I think the instrumentation is quite well-established and some improvements can be made through innovative designs and the use of state-of-the-art materials in the different components of the manifold. An example of this might be polymeric materials with enhanced chemical resistance. Another key point is software, which is particularly important for the applications involving wet chemistry assays. In this case, it is clear that current data processing and calculation for the generated results in commercially available software are not in the same stage of development as for other instrumentation that works with similar analytical outputs, such as chromatographic methods.

Hardik Shah: Liquid sensing errors are very common when working with plasma samples. This requires constant observation to minimize that error. Sometimes we see "pipette recognition errors" as well, find bubbles in the liquid delivery lines, or pipette tips are off from their intended targets. Improvements in those areas would be most welcome. Another area where improvement is needed is in software and method development. Methods are way too complicated and difficult for average users.

Alex Nisthal: Improvements in reliability and precision are always welcome. As for software and the general user experience, having two versions of the accompanying software could perhaps be useful. The advanced version would be the current version with many, many options to tinker with. The basic version might be useful for novice users who simply want to re-array liquids or something similarly easy. I believe Tecan has something like this now, but I haven't tried it. Second, improvements to separating the labware and robot vectors of different users and labs are needed.

FULL SPECTRUM DNA MEASUREMENTS IN LOW VOLUME SAMPLES, MICROPLATES, AND CUVETTES USING THE SPECTROSTAR NANO

Describe how ultra-fast, full spectrum DNA measurements are performed on the SPECTROstar Nano in three different volume formats: microplates, cuvettes, and low volume samples.

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INTRODUCTION:

Most substances in solution absorb light at a specific wavelength. When a defined amount of light is sent through a solution, a certain amount of light is absorbed by the substance. There is a direct linear relation between this absorbed light and the concentration of the solute (up to certain limits). This relation is shown in the Beer-Lambert law: $A = b \cdot c \cdot \varepsilon$ where b = pathlength [cm]; c = concentration of solute [mol/l or M]; and ε= substance-specific extinction coefficient [cm-1 M-1]. The reciprocal value of the coefficient at a 1 cm pathlength can be used to determine the concentration of nucleic acids without preparing a standard curve. Reciprocal extinction coefficients for dsDNA, ssDNA, and for RNA are widely known (50, 33, and 40 µg/mL, respectively).

The pathlength needs to be normalized to 1 cm in order to be used in the Beer-Lambert law. With the SPECTROstar Nano, cuvettes, microplates, and low volume samples (2 µl) can all be measured. Cuvette measurements are automatically normalized to the 1 cm length of the cuvette. In microplates the pathlength will vary depending on the liquid volume and well size. This can be normalized to a 1 cm pathlength by either: a) using a microplate with a defined pathlength and volume (the low volume LVis Plate has a pathlength of 0.5 mm when using 2 µL); b) using a pathlength correction where the volume and microplate dimensions are used in a mathematical algorithm; or c) using a known water peak value correction to normalize the data. With the SPECTROstar Nano, all three methods can be done.

EXPERIMENTAL CONDITIONS

Purified bacteria plasmid DNA was measured on the SPECTROstar Nano using a 1 cm cuvette, BMG LABTECH's LVis plate, and two microplates (96- and 384-well). The figure shows a full spectrum measurement of different concentrations of dsDNA in a 384-well microplate. Full spectrum analysis and BMG LABTECH's MARS data analysis software quickly and easily determines the DNA peak (260 nm), as well as the water peak (980 nm, not shown) and impurities at other wavelengths (230, 280, and 340 nm).

RESULTS

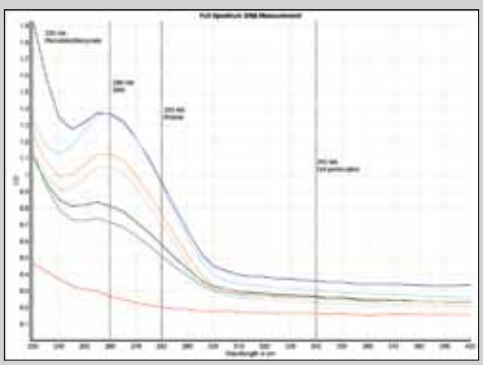
DNA samples measured in cuvettes, low volumes (LVis Plate), and microplates (96 and 384-well) all gave similar and reproducible results. Purity of DNA samples was determined using other wavelengths: protein (280 nm), phenolate or thiocyanate (230 nm), and cell particulates (340 nm).

CONCLUSION

Measuring DNA samples with the SPECTROstar Nano can be done in different formats: cuvettes, standard microplates, and low volumes using the LVis Plate. If there is enough DNA and only a few samples will be measured, cuvette measurements can be performed. For higher throughput a microplate can be used, or if sample volume is very limited, then the LVis Plate is recommended.

In addition, ultra-fast, full spectrum analysis allows for the samples to be measured only once. This greatly reduces reading times and errors due to measuring multiple times for multiple wavelengths.

Figure Legend: Full spectrum analysis on the SPECTROstar Nano is used to quickly measure DNA (260 nm) as well as impurities such as protein (280 nm), phenolate or thicocyanate (230 nm), and cell particulates (340 nm).



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1 Tap Run on the main menu

2 Select Method and tap Run

3 Load and inject sample