Pouring over liquid handling

Vivien Marx

A variety of liquid-handling methods are available for labs large and small. Selecting an approach is not just a matter of budget.

Bartenders and scientists have different workplaces and funding sources, but they share a concern about factors that affect the transfer of liquids from one container to another, such as dilution levels and materials loss. In the life sciences, a trickle of studies address the variability of liquid dispensing[1–3].

The paucity of studies is probably only a partial reflection of the knowledge about liquid handling. Like bartenders with their minds full of tips and tricks, scientists are probably keeping their flood of experiences to themselves for a number of reasons, not least because how they handle liquids is usually not the subject of their next grant proposal or paper.

Pharmaceutical companies test liquid-dispensing technology intensely to determine their "pet favorite" technology, says pharmacologist Sean Ekins, CEO of Collaborations in Chemistry. He advises drug-discovery researchers in industry and academia and has held positions at Pfizer and Eli Lilly and Company, and he co-authored a study published in *PLoS ONE* comparing pipette-based dispensing to acoustic dispensing[1].

Ekins hopes that he or others, perhaps a government lab, will one day perform a large-scale comparison of liquid-handling techniques for a variety of compounds and assays. "No one has done that so far," he says. "Isn't that astounding?" He and his colleagues have approached large academic screening centers he does not wish to name, but there is "not a lot of interest in doing the comparisons," he says.

Commercial and academic labs share an interest in enhancing drug-discovery approaches, especially at a time when many pharmaceutical companies are shedding research staff. There are liquid-dispensing systems aplenty. These days, however, purchases are "borne of necessity" to find a well-defined instrument to replace a missing or inefficient component in a workflow, says Jeff Jerman, who coordinates biology research and high-throughput screening at MRC Technology, a spin-out of the UK Medical Research Council (MRC). The projects he runs in preclinical drug discovery are similar to those once more common in pharma. Previously, Jerman worked on assay development and high-throughput screening at GlaxoSmithKline.

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Water has high surface tension and beads into a displaceable droplet. Liquids with lower surface tension are much harder to dispense.

The impact of variability in liquid dispensing is not to be underestimated, Ekins says. Many thousands of assays are performed in academia and industry; many papers are published every year that include assay data, which remains an understudied and largely unknown variable.

On Ekins' wish list is the hope that researchers—when depositing screening data in publicly accessible databases such as PubChem, a bioassay and compound repository run by the US National Institutes of Health—would indicate the type of liquid-handling mode used. To date, there is no such database or a large-scale comparative study to serve as reference. But scientists can consider the views of others about techniques.

Pipettes: thumbs up, thumbs down

In the life sciences, pipetting is everywhere; so are pipette tips, used in manual pipettes and in many large-scale automated liquid handlers. The most well-known type of pipette uses an air cushion to dispense a liquid. Less well known is the fact that air-cushion dispensing can deliver inconsistent results. Often, this inconsistency can be explained by manufacturing. "Many manufacturers use all different types of additives in the tip-making process," says Melinda Sheehan, product manager for liquid handling for Eppendorf North America.

These additives, such as slip agents, surfactants, plasticizers and bisphenol A, can all leach out—often irregularly—and

skew results from one replicate to the next. Leachates have received attention in the scientific literature and in industry, she says, pointing to a study by pharmacologist Andrew Holt of the University of Alberta and his team[2]. The scientists identified additives that leach out from some standard disposable plasticware and negatively affect experiments because the manufacturing chemicals turned out to be bioactive.

Sheehan says Eppendorf, unlike many other companies, does not use slip agents or release agents, which speed up the manufacturing of tips and tubes. Although the name may suggest otherwise, slip agents do not hasten dispensing. Rather, these chemicals are added to the polypropylene poured into a mold to reduce friction between the plastic and metal. In a lab, when plastic is heated, the pores in the material change size and chemicals can be released into the sample.

Even penny-pinching scientists should bear in mind how tubes and tips differ, she says. Polypropylene varies in quality. It is translucent, not transparent: clear tubes contain an added clarifier. Some tip manufacturers outsource production processes and may switch providers without buyers knowing.

Sheehan recommends that scientists inquire with suppliers whether the tips and tubes they are ordering are made with slip or release agents. "If they cannot confirm the absence, then maybe you should consider going with a manufacturer that can confirm the absence of these interfering agents," she says.

Jump off the tip

A number of companies, such as Eppendorf, Corning, Labcon, Sorenson BioScience and others, also offer low-retention tubes and tips. These plastics are designed to be less sticky for the liquids often used in assays. Water has high surface tension and beads into a droplet that can be readily moved. Many assays involve detergents that lower a liquid's surface tension and make it harder to move from the tip, she says.

Some low-binding tips are coated, which risks another chemical reaching the sample, Sheehan says. Her company's LoRetention tip uses no coating. Eppendorf has made proprietary molecular modifications to create liquid-repelling pipette material, allowing the liquid to "jump off the tip," she says.

Pipetting technique is another source of variability. Forward pipetting is best suited for water-based solutions, she says. In that technique, a researcher presses the plunger to the first stop, fills the pipette to the desired volume, and then dispenses the liquid by pressing the plunger to the second stop.

For viscous or volatile liquids, reverse pipetting is better, Sheehan says. A researcher presses the plunger to the second stop and fills the pipette tip with more liquid than the desired volume. To dispense the liquid, the user presses the pipette to the first stop, delivering the target volume. Residual sample can be discarded or returned to the stock container. In addition, she recommends slow pipetting to give liquids time to enter and exit the tip.

When giving pipetting seminars, Sheehan asks which technique a given group of researchers uses. "If it's a bunch of blank stares, then I say, 'Well, then you're doing forward pipetting technique.'" On average, around 10% of her audience tends to know the difference. If they are pipetting viscous liquids with forward pipetting, changing technique will help to achieve more consistent results.

Troubleshooting liquid-handling techniques becomes more important as assays involve smaller and smaller volumes. The importance of 1 µl retained in a tip after dispensing means more now than it did a decade ago, says Sheehan.

Positive-displacement dispensing technology, in which a piston slides down to force liquid off the tip, is always going to be better, more accurate and precise than any standard air-cushion system. "Always, hands down, for all liquids," Sheehan says, including aqueous, viscous or high–vapor pressure liquids. "But it is more expensive, so the scientist needs to make the choice." Researchers can select and also combine approaches depending on their compounds and types of experiments. But budgets are also part of the equation.

Stockbyte/PunchStock

Air cushion–based pipetting can deliver inconsistent experimental results. Manufacturing is one of many reasons.

Eppendorf

Scientists looking to cut costs should remember how tubes and tips differ, says Melinda Sheehan.

Joby Jenkins, who directs liquid handling for the instrument manufacturer TTP Labtech, says that accuracy and precision of tip-based dispensing is steadily improving right as assays increasingly move into the micro- and nanoliter range. One of his company's positive-displacement instruments, called the mosquito Crystal, is used by protein crystallographers, and other areas are emerging that call for dispensing of small liquid volumes.

Jenkins notes that biologics in drug discovery are often stored in aqueous or glycerol stock solutions. Low-volume liquid dispensing in these cases can be particularly affected by surface tension. In his view, positive displacement technology handles these liquids well.

High-throughput screens

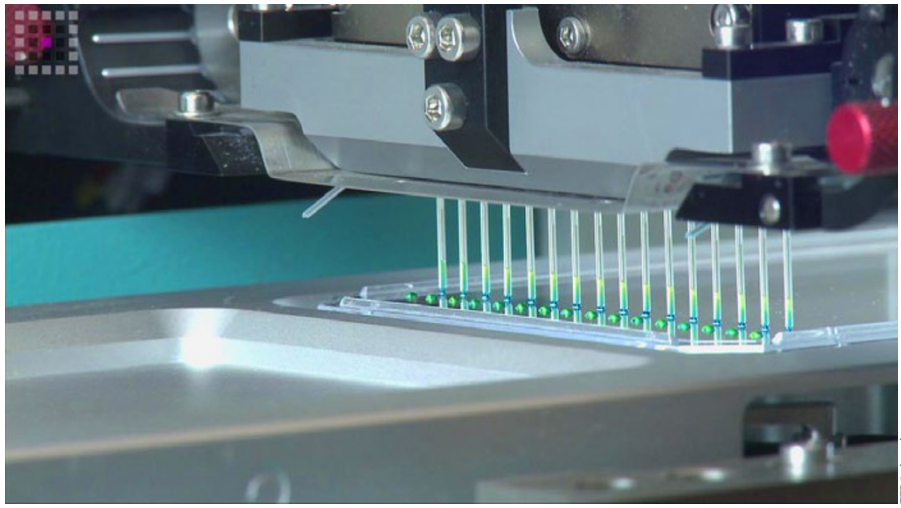
Automation scales up assays that are used, for example, to identify a compound that inhibits an enzyme in a desired way. Drug-discovery scientists interviewed by *Nature Methods* explain that typical assays involve thousands or millions of compounds. Each assay measures effects on the activity of an enzyme implicated in a disease. Compounds are eliminated for various reasons—they may show no activity or too much.

Scientists perform assays at varying concentrations of the compound as they search for the one that will inhibit the enzyme at nanomolar or perhaps picomolar concentrations. Then, medicinal chemists finesse some of these drug candidates. Not all assays are of this type—some have to be adapted to precious biological materials, such as stem cells.

High-throughput assays require plenty of liquid dispensing. Compounds dissolved in dimethylsulfoxide (DMSO), a common solvent, must be moved to assay plates quickly and on a large scale. Some approaches also use serial steps of dilution. Views differ widely on these steps, which is why some instrument manufacturers choose techniques such as acoustic or inkjet technologies that avoid serial dilution.

The recent *PLoS ONE* study showed, as some commenters saw it, a disturbing difference between two liquid-dispensing approaches[1]. One method seemed to deliver completely different results on the biological activity of the dispensed compounds, which were potential anticancer agents.

The study authors compared acoustic liquid dispensing and pipette tip–based dispensing using data from AstraZeneca patent applications for a number of small-molecule tyrosine kinase inhibitors. The data from acoustic dispensing led to calculated pharmacophore models that are, the authors suggest, more representative of actual crystal structure data, a result indicating that acoustic liquid dispensing is more reliable for drug discovery than using pipette tips. Pharmacophores guide the understanding of receptor binding characteristics and aid medicinal chemists in improving binding traits.



TTP Labtech

In positive-displacement dispensers, a piston pushes liquid off the pipette tip.

Touchy subject

Ekins, a coauthor of the *PLoS ONE* study, says that big differences between techniques can have nightmarish consequences. If a method to obtain preliminary data is flawed, "it could send you off in the wrong direction." Scientists need to assume that any material touching their compounds can have an effect.

S. Ekins

Big differences between liquid-handling techniques can have nightmarish consequences, says Sean Ekins.

With plastic tips, many factors can influence experiments, so it is important to identify the hitches and account for them, he says. One factor is physical-chemical interaction between the compounds and the plastic.

The compounds are often greasy, which makes them stick to the plastic tips. Hydrophobicity partially explains the variation between liquid-handling techniques observed in the study, "but I don't think it's the complete factor." Ekins says. "We weren't going to be able to tease that out with the few compounds that we had."

Acoustic dispensing moves a liquid without direct contact but with a focused beam of sound. "If you focus it right at the meniscus—the surface of the liquid—you can make this droplet shoot off with very, very high precision and accuracy," says Joe Olechno, a researcher at Labcyte, a company that sells its Echo series acoustic dispensers to pharmaceutical industry and academic labs. He is a co-author of the *PLoS ONE* paper[1], which, in his analysis, showed that pipette tip-based systems could be wrong by 200-fold compared to acoustic dispensing.

In the Labcyte instruments, a droplet is ejected from the fluid surface and flies up to its destination—a well of an assay plate, for example, or a microscope slide. The wells of the source plate in the instrument face upward; the destination plate's wells face down. The inversion helps the destination plate to catch the droplets when they shoot upward, Olechno says.

Labcyte's idea of a "nozzle-less nozzle," as Olechno says, was to avoid complete or partial clogging of liquid-handling nozzles. Having clogged nozzles also risks spraying liquid into the wrong assay wells or delivering differing liquid volumes. Acoustic dispensing avoids those issues, and it does not leave remnants of liquid at the pipette tip's end, he says. It also saves the cost of pipette tips, which sometimes also must be disposed of as hazardous waste.

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Variability due to liquid dispensing affects experiments of all sizes.

Although early models of acoustic liquid dispensing could move only DMSO, technology development has progressed to allow dispensing of other types of liquids, including aqueous, glycerol-containing and soapy solutions, as well as mixtures of these liquids. Plates can also have different liquids in each well. The Labcyte system measures each well acoustically, capturing surface tension and viscosity, and can adjust for the amount of energy required to complete a transfer, he says.

Commenting critically on the *PLoS ONE* paper, Jenkins notes that the study does not offer detail on the tip-based dispensing used. Their use of data from AstraZeneca patents for the study meant, indeed, that Olechno and his colleagues did not know which kinds of tips were used in the company's experiments. But given that it is a pharmaceutical company, "you would think they would be doing best practices, especially if they put it into a patent," says Olechno.

Jenkins says a number of factors can explain the differences between acoustic and tip-based techniques, including shifts in compound potency, variation between batches of synthesized compound, the consumables used, the number of times tips are changed throughout the process or leachates from the plasticware in which compounds were stored. A compound can 'crash', or precipitate out of solution. This crashing can happen when intermediate dilutions are

Labcyte's acoustic dispenser ejects a droplet from the fluid surface.

performed with an aqueous diluent to reduce DMSO concentration, he says.

TTP Labtech liquid handlers use positive-displacement pipetting to move liquids and dilutions in 100% DMSO in 'assay-ready' nanoliter volumes, which, he says, avoids this crashing situation. Each pipette has a stainless steel piston that pushes liquid out of the pipette accurately, which, Jenkins says, avoids clogged tips, leaves behind "negligible" amounts of liquid, or 'dead volumes', in the pipette and works equally well for all liquid types, independent of viscosity, surface tension or environmental conditions.

Olechno agrees that pipettes with pistons do "a great job" in transferring the correct liquid amounts. But scientists care more about the material dissolved in the sample. If the volume is correct but all of the compound is stuck to wells and tips "that's what screws you up," he says. Addressing the price tag of acoustic dispensing systems, Olechno says that reducing assay volumes and eliminating tips can save much money for labs.

Try them all

Acoustic dispensing is "a very valuable technology" in commercial drug discovery's high-throughput screening, says Alastair Binnie, vice president of research information technology and automation at Bristol-Myers Squibb (BMS). And "in some critical applications, it's superior to pipette-based dispensing."

Binnie introduced acoustic dispensing to BMS when Labcyte was still a start-up, more than a decade ago. At the time, he says, he saw a technology gap for low-volume sample transfer. High-throughput screens called for a robust and fast way to move compounds stored in DMSO from a stock plate to an assay plate.

Typical screens, which can involve as many as 1 million compounds in conventional 96-well plates and an assay volume of 100 μl, were "prohibitively expensive in standard reagents and labware, and also required infeasible quantities of precious, custom-made proteins," Binnie says.

"We'd tried various technologies based on conventional pipetting, but they were expensive, slow, inaccurate and prone to contamination even after multiple time-consuming washing steps," he says. The team tried pin-based dispensing, but it was inaccurate and also needed washing. No technique was sufficiently robust, clean, cheap and fast enough for high-throughput screening.

When acoustic dispensing came on the scene, with the promise of no washing, no tips and no moving parts, he saw its promise. Early prototypes confirmed this technique, he says, as a "missing link" to help miniaturize high-throughput screening.

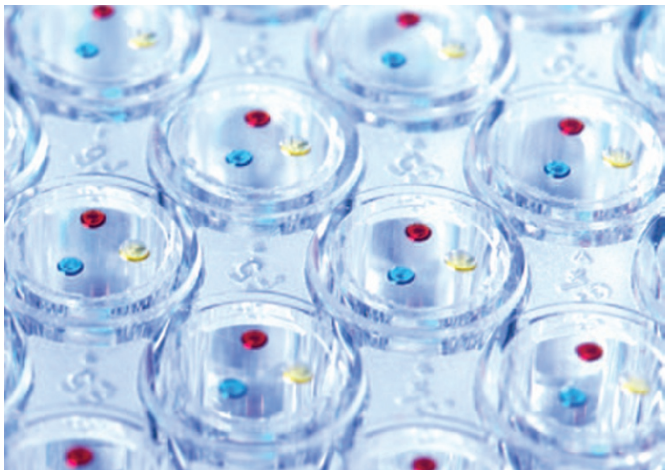
Acoustic dispensing instruments from Labcyte are now "our workhorses" as a cost-effective approach for many assay formats, says Binnie. And, he says, its advantage over pipetting is that it is "robust, accurate, fast and clean low-volume dispensing, for situations where serial dilution is not an option."

Binnie wonders, however, whether the differences in dispensing methods are so profound that computational models derived from pipette-based data might be seriously misleading. The *PLoS ONE* study uses a small data set and includes little detail about experimental methods, making it hard to know for certain whether the only variable is the difference between acoustic and pipette-based dispensing, he says.

Some variation is always expected because of the many differences between the assay conditions. And, Binnie says, "if it's really true that acoustic dispensing yields such dramatically different data over pipette serial dilution, to the point where pipette data can't be trusted, then I just think we'd have noticed it by now." It has become routine, he says, for a compound to be first tested in high-throughput screens using acoustic dispensing, followed by pipette-based serial dilution.

Hidden assumptions

Another part of reducing variability in liquid handling is quality control of instruments and tips, says Jenkins. Artel's multichannel verification system, which he calls a "gold standard," is able to "independently verify the accuracy and precision of each tip of an automated liquid handler in one rapid experiment." His customers usually use Artel



TTP Labtech

Liquids are sometimes dispensed onto inverted plates. Crystals grow in hanging droplets in protein crystallography.

instruments or systems they developed in house to detect any dips in performance of TTP Labtechs's instruments.

Tracking all the materials that touch a compound calls for much diligence. "Don't take the little things for granted and question assumptions," says George Rodrigues, who directs scientific activities at Artel, which manufactures volume-measuring systems that work on pipette tip–based instruments and acoustic dispensers.

Many hidden assumptions are at work in labs—for example, when diluting and performing dilution calculations, scientists might assume that molecules will not partition to surfaces. Dispensers might not be properly calibrated or solutions can be incompletely mixed, Rodrigues says.

The *PLoS ONE* paper and others like it make it essential to find the "root cause" of liquid-handling variability and might be hinting at chemical partitioning onto pipette tip surfaces, but, he says, "I'd like to see the proof of the physical cause."

Rodrigues believes that the typical scientist underestimates the amount of variability of simple laboratory tasks. He sees more awareness of quality control in the pharma industry's regulated environments than in academic labs. But there is variation in industry, too, due in part to individual lab managers and the culture in a given organization, he says.

Digital dispensing

Recently emerging in the benchtop market niche, next to expensive automated systems and manual pipetting, is a pipette-less system developed by engineers at Hewlett-Packard (HP).

The engineers expanded on inkjet printer technology to design a dispensing head that has multiple nozzles fabricated onto a silicon chip, says Kevin Peters, the scientist who co-invented the instrument and manages life-science business development at HP. The designers of the HP 300 Digital Dispenser wanted to address issues in inkjet-type technologies that have fared poorly in the past.

In this instrument, a bubble displaces the liquid. As Peters explains, the design involves routing fluid onto a chip. Particulates that could clog nozzles are filtered out. A droplet is created by flash heating a solvent, forming a gas bubble. It is the gas bubble that pushes an unheated liquid droplet out the nozzle. The gas bubble collapses and refills itself.

In 2011, HP began collaborating with Tecan, a manufacturer of liquid-handling instruments. Both companies distribute, service and support these HP instruments.

The HP system dispenses directly to avoid one of the great headaches in liquid handling: titration, in which a concentrated liquid is diluted over several steps and in which issues abound, such as pipetting inaccuracies, sore thumbs, limits to solubility and loss of material to vessels and pipette tips used. Acoustic dispensing and direct dispensing each addresses these issues in its own way. The HP instrument avoids serial dilution and 'prints' picoliter droplets directly into assay wells.

As MRC Technology's Jerman explains, he and his colleagues use liquid handling for small-molecule drug screening and to prepare compounds with microtiter plates. They traditionally use tip-based systems, which, he says, "offer broad brush in terms of functionality but fail somewhat in the low-volume end." To fill that gap, he chose digital dispensers, a decision "based almost solely on upfront costs." Unlike pharma, he says he and his colleagues have the "luxury" of not being linked through multiple sites around the world, so they can avoid standardization of hardware and process as well as committee decisions on instrument acquisitions.

He added the HP system to the MRC Technology workflow between high-throughput tip-based systems and the often "hand-crafted" assay plate layout. A drawback of the HP system is that it cannot be used to dispense aqueous solutions, says Jerman. Its advantages include relatively low up-front cost, a small footprint, flexibility in assay and compound layout, ease of use and a "massive" saving of compound that is otherwise lost to dead volume in dispensing.

Labcyte's Olechno is concerned about the fact that ink-jet methods use heat to dispense analytes. Although it is usually not a problem for small molecules, heat can denature enzymes transferred in this fashion. Clogged nozzles or solvent that evaporates and leaves a drug candidate behind could also be problems, he says.

Peters says that the HP dispense heads have micromachined filtration structures that avoid clogging. Single-use dispense heads sidestep the need for cleaning or calibration. He also says that less than 0.2% of the volume is flash heated for only a microsecond, which drug-discovery customers are not finding problematic.

Facing reality

Faced with a multitude of choices for liquid dispensing, scientists in pharmaceutical R&D may have the option of using multiple methods side by side. Other labs' financial constraints might not permit that comparison.

Acoustic systems are not about to push pipette-based systems out of the market, says Eppendorf's Sheehan. One main reason is that acoustic systems are cost-prohibitive for most labs, and another is the host of liquid-handling variables.

Working in an academic lab before her employment at Eppendorf, Sheehan had a tendency to critique the pipetting techniques of her co-workers. They teased her as "the pipette police." Beyond the realm of name-calling, it seems that evaluating and policing liquid-handling techniques of all types is an advisable approach to keep experiments flowing on a large or small scale.

Corrected after print 13 January 2014.

1. Ekins, S., Olechno, J. & Williams, A.J. *PLoS ONE* **8**, e62325 (2013).
2. McDonald, G.R. *et al. Science* **322**, 917 (2008).
3. Butendeich, H., Pierret, N.M. & Numao, S. *J. Lab. Autom.* **18**, 245–250 (2013).

Vivien Marx is technology editor for *Nature* and *Nature Methods*.

Erratum: Pouring over liquid handling

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In the version of this article initially published, the text stated that Toby Jenkins directs liquid handling for instrument manufacturer TTP Labtech. This name was misspelled; the correct name is Joby Jenkins. The error has been corrected in the HTML and PDF versions of the article.