SERIAL vs DIRECT DILUTION

Time to apply new thinking to IC_{50} determination and dose-response analysis?

By Dr John Comley

The serial dilution method is standard practice in the preparation of dose-response series for IC_{50} determination. However, it is well recognised that inadequacies in the liquid handling or mixing technique will affect the dilution ratio and hence the compound concentration and any errors will be compounded during each successive serial dilution, mix and transfer. A recent poll of end users ranked better precision, particularly at lower drug concentrations, and the reduction in compound precipitation as the improvements in dose-response analysis they most desired. In addition, it is now suspected that hydrophobic compounds may be lost from solution during aqueous serial dilutions and absorbed to intermediate plastic surfaces. This in turn adds to concern over the reliability of the results generated and the extent to which they are a true reflection of the potency of the compounds being evaluated. As part of the general drive to enhance the quality of screening data generated researchers are investigating strategies based on the direct dilution of micro-volumes of compound (ie on a volumetric basis). These investigations have been aided by the availability of low volume dispensing systems with good precision at low nL dispense volumes and a relatively wide dynamic range. Some groups are now reporting that IC_{50} values of compounds tend to be lower (more active) when the concentrations are made via direct dilutions. It is increasingly evident that direct dilution has a future role to play in dose-response analysis and where acoustic droplet ejection is preferentially deployed additional benefits will be derived in terms of reduced waste stream generated, less source material used and no cross-contamination.

The uptake and use of nanolitre dispensing within drug discovery is now widespread, with nearly two-thirds of all groups involved in dispensing activities having access to nanolitre (nL) volumes today and most of the remainder considering or planning to acquire a nL capability in the future (**Figure 1**)[1].

Although plate replication and compound reformatting still represent the main applications for nanolitre dispensing in drug discovery today, there is increasing use being made of cherry picking low volumes both for, or directly used in the set up of, IC_{50} or dose-response analysis (57% now using) (**Figure 2**).

Most of the nL dispenser offerings available today (eg Beckman PicoRAPTR™, CyBi®-NanoJet or Labcyte® Echo®, etc) are standalone and typically have only a small amount of automation built into them. However, in order to fully automate the main applications of nL dispensing it is evident that users will need to integrate these systems with other components (eg input or output plate stacking/hotel; robotic plate handler; bulk reagent dispenser; and controlling software) and possibly other non-essential plate processing peripherals (eg incubator; mixer; lidder/delidder; sealer; reader, etc). When interest to purchase a standardised fully automated nL dispensing system for the main applications of nL systems was investigated it was apparent that most end users would like to access a system that is flexible enough to be able to perform multiple applications, ie compound reformatting combined with dose-response and IC_{50} preparation in the same system (**Figure 3**).

Serial dilutions

The generation of dose-response curves requires the preparation of solutions of compound at various concentrations over a wide range, often covering six logs of magnitude. These concentrations have more traditionally been made by a serial dilution technique in which a stock solution of the active compound of interest, typically in 100% DMSO, is cherry picked. This may take the form of aspirating an aliquot from a selected well in a source library plate or by punching out a pre-aliquoted volume stored in mini-tube. In either case, the aliquot is then diluted with aqueous diluent or buffer at a fixed ratio (eg 1 into 3) and mixed thoroughly, often by repeat aspirate and dispense cycles within the pipette tip. An aliquot of diluted drug is then removed and added to a volume of new aqueous diluent at the same ratio as the first dilution. Typically dilutions are performed in adjacent wells along the row or column of the plate, with successive serial dilutions made until the dose response range required in the series is achieved. When this intermediate plate is

Figure 1: Uptake of nanolitre in drug discovery dispensing today

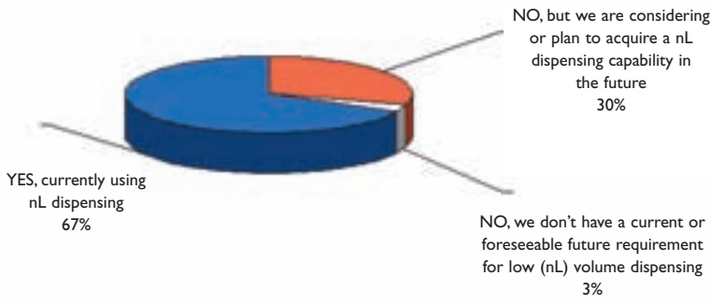
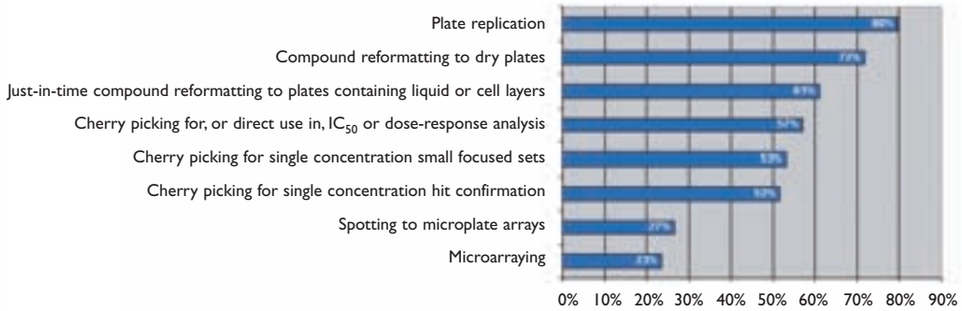


Figure 2: Main applications of nanolitre dispensing in drug discovery

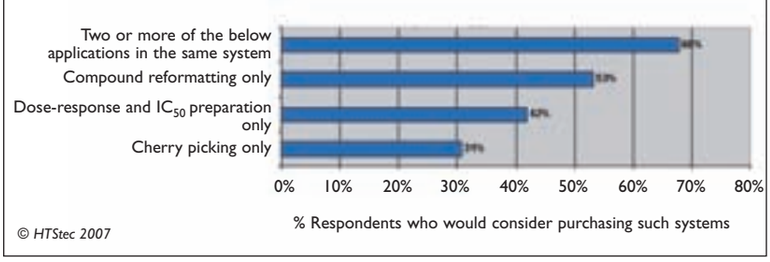


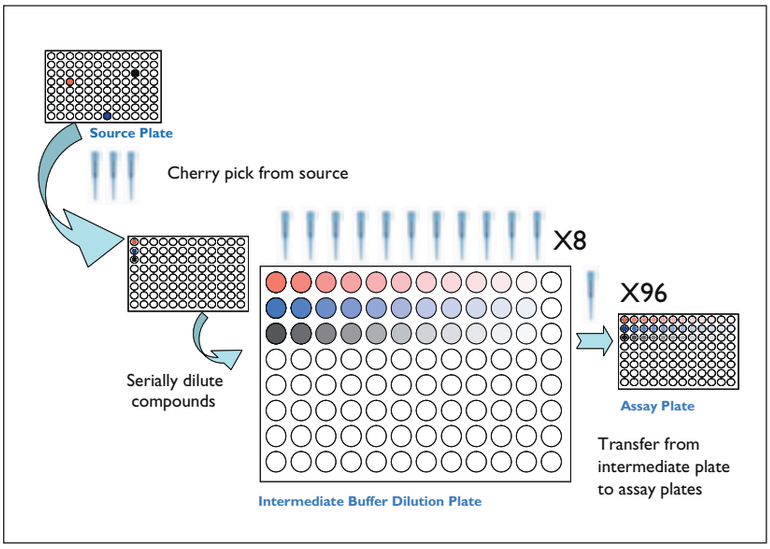
complete, it can be reformatted or replicated to multiple assay plates. The key point with serial dilution being that it has been standard practise to dilute drug stocks that were initially prepared and solubilised with 100% DMS0, with aqueous solutions to minimise the final DMSO concentration in the assay. In addition, it is usual to prepare relatively large (µL) volumes of a dilution series in a separate intermediate plate or series of tubes, distinct from the ones in which the assay(s) will be undertaken, contributing to the overall greater use of compound than is necessary for the setup of multiple assay dose-response curves. (**Figures 4** and **Figure 5**).

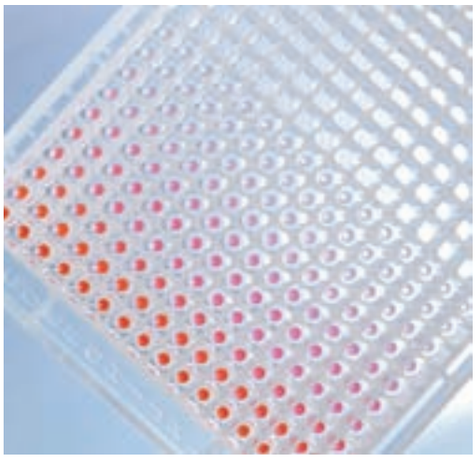
Direct dilutions

The alternative approach to serial dilution is the direct dilution of micro-volumes of compound (ie on a volumetric basis). In this case the volume actually dispensed is directly proportional to the amount of compound required to give the desired concentration in the chosen final assay volume. Although the concept of direct dilution has been around and discussed for many years, the recent availability of low volume dispensing systems able to deliver with better precision a minimum volume dispense in the region of 1 to 10nL and a relatively wide dynamic range, has resulted in the approach finally being investigated more widely, with the practicalities of how the technique might be routinely implemented considered and the benefits realised. As with most of the recent changes adopted in drug discovery processing, many improvements have been driven by the desire to enhance the quality of data generated. In the case of dose-response analysis, the concern has been the reliability of the results generated and the extent to which they are a true reflection of the potency of the compounds being evaluated. The

Figure 3: Interest in purchasing a standardised fully automated nL dispensing system for specific applications







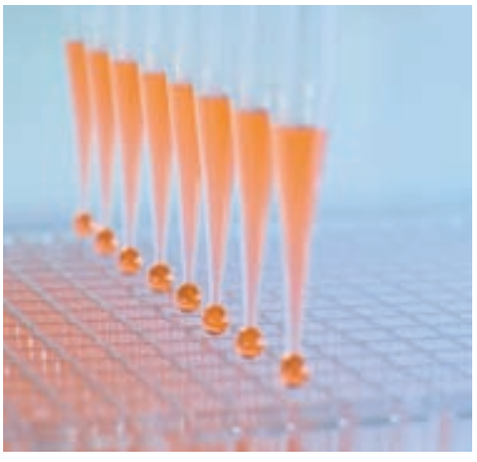
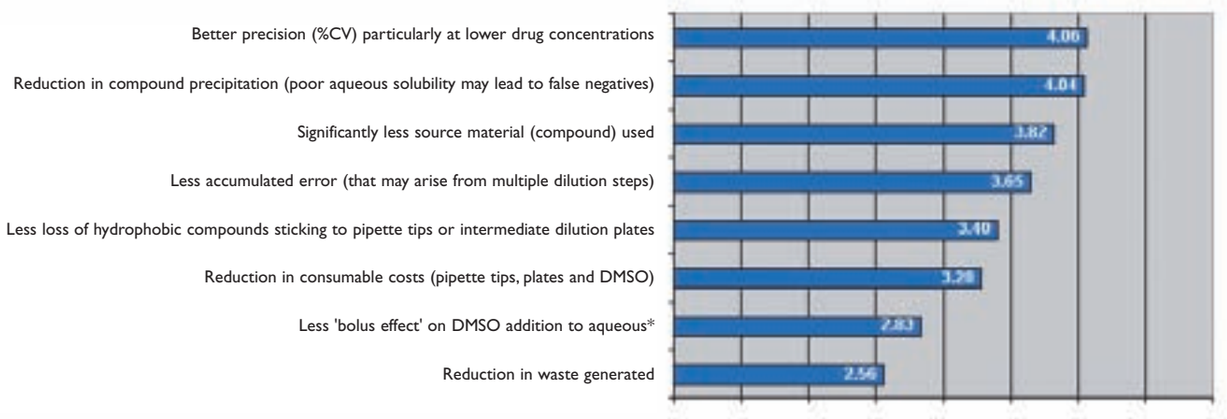


Figure 4 (above)
Traditional serial dilution technique used in IC_{50} and dose-response analysis

Figure 5 (left)
Serial dilution of dye down the rows of microplate

Figure 6: Improvements desired when undertaking dose response analysis today



** Notes on the 'Bolus Effect' – Acoustic droplet ejection adds solutions of drug candidates to cells with the cell-containing microplate in an inverted position. This leads to a major benefit for cell-based analyses. Typically when DMSO solutions are added to microplates containing cells, a bolus of DMSO containing test compound sinks through the surrounding cell medium and is in contact with the cells at far higher concentrations than the final equilibrium concentration. This can lead to cell damage or death and make it difficult to determine the actual effect of the test compound. When DMSO is added to an inverted plate, the DMSO spreads out across the meniscus and only after the plate is returned to its upright position does the DMSO begin to drop through the solution. By this time, the DMSO has spread over the entire meniscus and the distributed solution diffuses smoothly through the cell medium.*

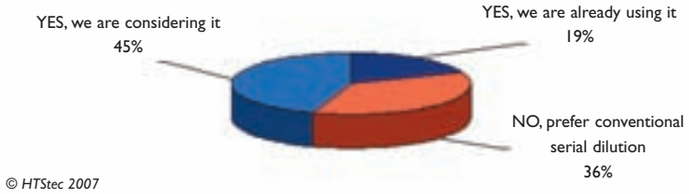
relative ranking of the importance of improvements desired when undertaking dose-response analysis today are presented in **Figure 6**. This shows that better precision (%CV) particularly at lower drug concentrations and the reduction in compound precipitation (induced by poor solubility in aqueous solution which could lead to false negatives) were ranked as the improvements respondents most desired. Interestingly, progress towards addressing this entire list of improvements could be expected to be derived from the application of direct dilution approaches. With this in mind, interest in performing automated IC_{50} and dose-response analysis by direct dilution was investigated[1]. It was found that around 1 in 5 (19%) current users of nL dispensing are already using direct dilution strategies today and a further 45% are considering it (**Figure 7**), suggesting that the expectation is that tangible benefits will be derived from the direct approach.

Vendor updates

In the following vendor updates we will learn about some of the current approaches to serial and direct dilution for IC_{50} determination and dose-response analysis. In particular, we will examine the role that low volume and nanolitre dispensing can make to such activities; whether positive displacement micropipettes or piezo dispensing holds the key to direct dilution; how new automated, including microfluidic, systems could significantly improve such activities; where new tracking methodology is being applied to enhance the quality of dose-response data; where optimisation of dispensing and mixing parameters has yielded faster processing and superior data; and whether the IC_{50} is affected by the dilution strategy.

Artel (www.artel-usa.com) has enhanced its MVS® (Multichannel Verification System) to provide laboratories with the first standardised technology to verify the accuracy of dilution ratios in serial dilution protocols. This new capability is essential for drug discovery and other laboratories that rely on data generated from dilution-based liquid delivery procedures. To generate a proper serial dilution methodology, during which a sample solution is

Figure 7: Interest in performing automated IC_{50} and dose-response analysis by direct dilution



diluted across a microtiter plate, accurate and repeatable volume transfer and efficient solution mixing are critical. It is also imperative that the sample concentration in the initial solution be known so that the decreasing concentrations of sample across the plate can be correctly determined. Because sample concentration is directly affected by starting concentration and the amount of transferred volume, an inaccurate liquid handler can cause unseen, compounding errors in serial dilution applications, which can void the integrity of the dilution assay. Previously, the only option available for laboratories to measure dilution ratios in serial dilution protocols were home-brewed methods requiring internally developed sample solutions. This time-consuming process does not allow for inter-laboratory comparability or traceability. By contrast, the MVS technology and range of standardised solutions allow for rapid verification of dilution ratios and provide traceable results for compliance and reproducibility. The MVS verifies target volumes via ratiometric photometry, which uses the absorbance values of two proprietary dye solutions to calculate dispensed volume. The enhanced MVS now provides traceable measurements for single- or multiple-point dilutions to a concentration endpoint ratio of 1:2000 of the starting material (**Figure 8**).

Figure 8: Artel's MVS® (Multichannel Verification System)

Beckman Coulter's (www.beckman.com) PicoRAPTR™ (Picolitre Rapid Transfer Robot) offers ultra-low volume aspirate and dispense operations. The system consists of an eight tip piezo head with eight independent fluid paths and dual plate carrier stage which allows for high speed aspiration (1µL to 100µL) and dispense (1nL to 100µL) volumes. The ultra clean sonication wash, inert fluid pathways, precise spatial positioning and 100% DMSO compatibility enables high-quality dose response curves using direct or serial dilution. With DMSO correction of liquid and the extended pipetting dispense range you are able to achieve a 5 log concentration giving a percentage bias within 10% and precision %CV of less than 10%. 90% of the aspirated solution is available with using an air gap resulting in reagent cost savings and allowing for the filling of an entire microplate from a single aspirate. The system will accommodate source plates of 96, 384, 1536 well microplates and destination plates of 96, 384, 1536, 3456 well microplates. Beckman Coulter also offers selective tip loading for the Biomek® FXP Laboratory Automation Workstation multichannel pipetting head. This allows for serial dilutions to be performed by configuring the loading of tips for any row(s) or column(s) the user needs to work with (**Figure 9**).

Figure 9: Beckman's PicoRAPTR™ dispenser

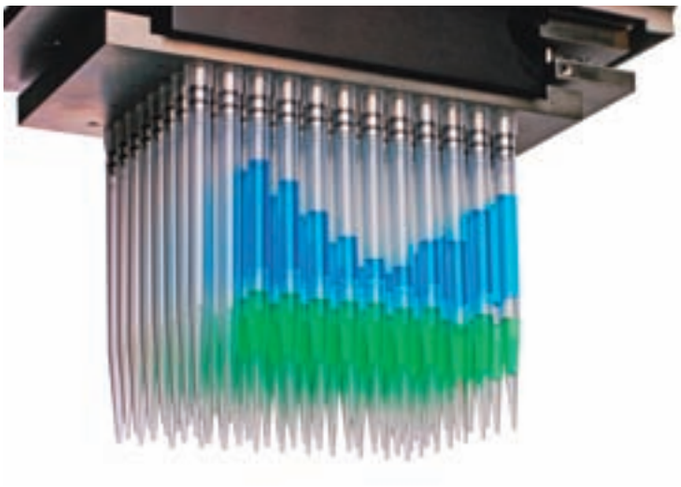


Figure 10
Variable volume aspiration on the Caliper Life Sciences Sciclone i1000

The Intelligent Sciclone i1000 from **Caliper Life Sciences** (www.caliperLS.com) offers for the very first time independent pipetting control over each channel in a 96 channel pipetting head meaning that each channel can pipette its own unique fluid volume. The heart of the system is the real-time feedback, self-diagnosing and adaptive control enabled by the proprietary MEMS micro flow meters built in to each of the 96 independent channels. One hundred and nine micro-processors monitor performance parameters in real time and facilitate 'on the fly' adjustments to compensate for variations like temperature and viscosity. In addition, each channel has the ability to pipette across an extremely wide dynamic range from 5 to 1,000 microlitres. With this capability, researchers can do one-step plate normalisations, IC_{50} experiments, serial dilutions and other multiparameter experiments at very high throughput. For example, to perform a single step serial dilution, a volume file in an Excel format of 96 volumes of dilution buffer is given to the head followed by a file of 96 complementary volumes of target compound. Two aspiration steps, first of the buffer then the target compound, are followed by a single dispense step to create a plate with 96 different concentrations of the compound with a dilution factor of 1:200 across the plate. If required, this plate could be further diluted in a second step for a total dilution range of 1:40,000 (**Figure 10**).

For dilution operations in the low volume range **CyBio** (www.cybio-ag.com) provides two dedicated instruments. For serial dilutions the CyBi®-DiluSpro can be used, for direct dilutions the CyBi®-NanoJet is the instrument of choice. The CyBi®-DiluSpro is an economically priced 8-channel pipettor which is perfectly designed for the automatic preparation of serial dilutions. The large volume range (250µL head: 0.5-250µL; 25µL head: 0.1-25µL) allows the execution of serial dilution as well as the direct dilution in a special range (1:500) depending on the dispense volume. The CyBi®-DiluSpro is a modular device. The user has the choice of three different carriage systems (4-, 5-place linear carriage and 10-place circular system), three different tip types for a single device and two different software packages (either optimised programs/graphical user interface or a Scripting Studio for creating own programs) depending on the requirements of the customer (**Figure 11**). The

Figure 11 (above): CyBio's CyBi®-DiluSpro

Figure 12 (left): CyBio's CyBi®-NanoJet

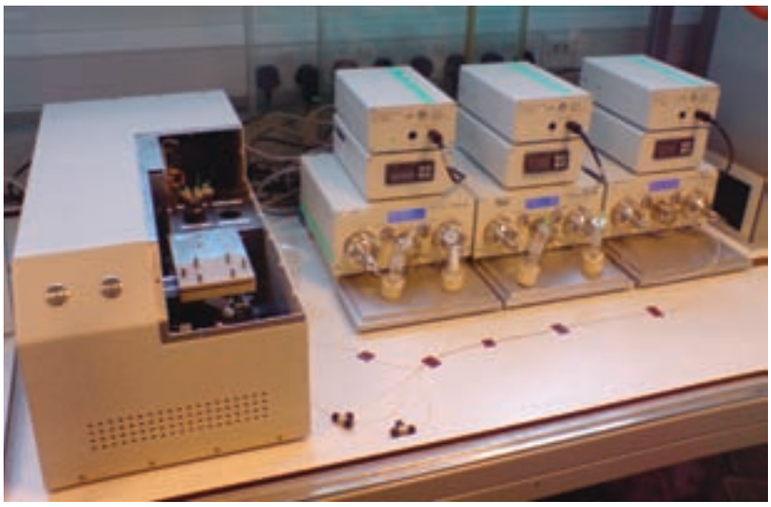


Figure 13: Genapta's direct dilution IC_{50} engine. Compounds are introduced as single samples in the milli-molar regime. They are diluted and assayed automatically by the system without the intervention of any external sample handling or automation. The device is capable of producing a 100-200 point IC_{50} curve in 30 minutes using just a few microlitres of reagent. (*Photo courtesy Ley Group, Dept of Chemistry, Univ of Cambridge*)

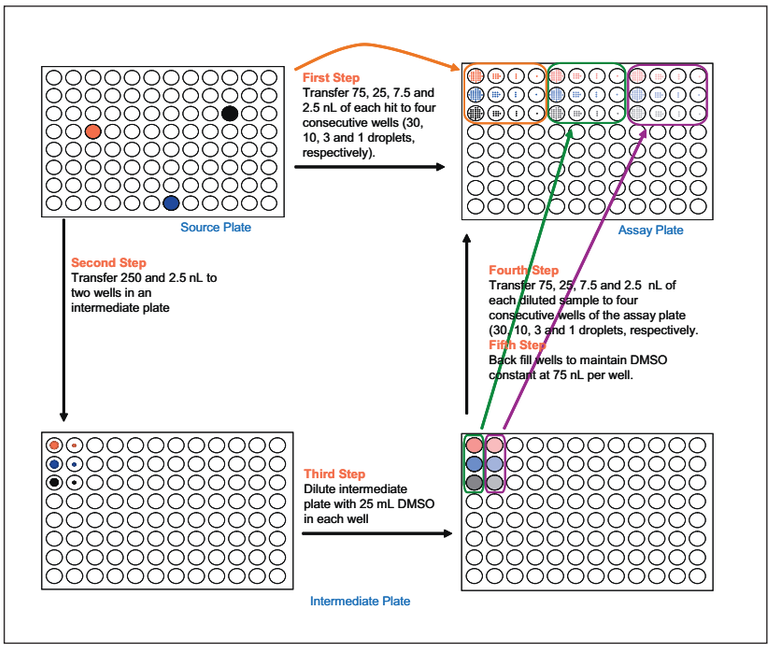


Figure 14: Acoustic droplet ejection (ADE) allows for an efficient direct dilution technique. Active compounds are cherry-picked from source plates at four volumes directly to an assay plate (Step 1). These same compounds are transferred to an intermediate plate at two volumes and diluted with 25µL DMSO to provide dilutions of 100:1 and 10,000:1 (Step 2). Dilution directly into DMSO eliminates the potential for compound precipitation and reduces absorbance to the plate (Step 3). Each of these diluted solutions is transferred to the assay plate in four volumes as indicated (Step 4). DMSO is back-filled using ADE to maintain an equal amount of DMSO (75nL) in each well (Step 5). A 10µL assay will have a total concentration of 0.75% DMSO

CyBi®-NanoJet is a very flexible, bulk dispensing instrument operating in the low volume range from 50nL to 20uL per shot. The pressure/valve based system can handle up to 16 independent reagent lines and can access every well individually in any SBS plate format. Reagents, cells or beads can be dispensed with a nearly unmatched precision. Direct dilutions can be performed using the instrument stand alone by adding two different reagents into single wells, which allows dilution rates of more than 1,000 (eg 50nL in 60uL). Depending on the operation mode the CyBi®-NanoJet can be used as a flexible assay workstation or as a fast HTS assay processor (**Figure 12**).

Genapta (www.genapta.com) has been designing and building direct dilution potency systems in collaboration with a major pharma partner for the past five years. Its current product development (the PIC50 system) is for enzymatic assays, and later in 2007 will pick up cell based assays with the instrument being released on to the open market Q3, 2007. Direct dilution, rather than a well by well discrete approach, has mostly become a reality because of the availability of high quality pumping systems. In particular the advent of nanoscale HPLC systems, has allowed the ability to control liquid flows down to a few nL per minute, which in turn enables detailed external control of the mixing conditions on a microfluidic chip. Thus it is possible to create a dynamic and varying concentration gradient against which to perform a competitive binding assay by controlling flow rates rather than trying to dispense and mix metered volumes of reagents into a well. This approach has a number of advantages that include repeatability and ease of set up. In addition to the flow control, a miniaturised spectrometer is used to assay the reaction. Together these elements form a miniaturised-screening platform. As the system takes a single slug of compound that is diluted as it is measured, the use of microwell plates as a carrier medium is greatly reduced, and the device needs little or no external automation to generate potency data for the medicinal chemist. As such it allows this potency to be generated alongside basic toxicity in timescales of hours rather than weeks. Such a reduction in assay time increases the potential for iteration during discovery 10-fold, allowing many more elements in a candidate series to be characterised at the crucial later stages (**Figure 13**).

Acoustic droplet ejection (ADE) on the **Labcyte®** Echo® (www.labcyte.com) has been used to investigate direct dilutions of small volumes. Using the

protocol outlined in **Figure 14**, 12-point direct dilutions over six orders of magnitude can be prepared. Recently researchers at Bristol-Myers Squibb have reported[2] that the IC_{50} values of compounds tend to be significantly lower (more active) when the concentrations are made via direct dilutions of small volumes than when the concentrations are achieved with serial dilution (**Figure 15**). It has been postulated that this was due to compounds adsorbing to the solid surfaces of the dilution plate wells and to the pipette tips used to transfer the fluids. Hydrophobic compounds suffered the most loss during serial dilutions. Recent work using mass spectroscopy to measure the concentrations of solutions has provided independent analytical support to the hypothesis that significant amounts of compound are lost during serial dilution. The actual concentration of analyte in serially diluted solutions can be far less than anticipated, and may cause false negatives in primary screening tests for potency as well as inaccurate IC_{50} values. In addition to the loss of high value hits, these false negatives also create difficulties for medicinal chemists and chemo-informatics programmes attempting to draw conclusions about structure-activity relationships. The recently launched Labcyte® POD™ 810 plate assembler (**Figure 16**) robotically integrates an Echo® liquid handler for ADE with plate storage, a bulk filler for reagents or diluents, and software designed to optimise direct dilution techniques for dose-response curves. The POD plate assembler takes source plates and optimises the throughput of ADE to provide destination plates ready for assay or further storage.

The need for high quality dose response data continues to drive the demand for serial dilution, especially in the area of low volume dispensing. This is reflected in a continued level of interest for serial dilution functionality in the fully integrated liquid handling systems that **RTS Life Science** (www.rtslifescience.com) produces. A system recently developed for one client utilised twin Velocity11 VPrep liquid handlers installed within an automated robotic cell. One of the key challenges that RTS had to overcome was the need to dynamically generate custom plate well information so as to ensure that the correct serial dilution was performed for specific plates being processed through the system. Key to this was the tight integration afforded by RTS' d-Sprint™ and Sprint™ software platforms. A custom developed Sprint™ device driver allowed the dynamic population, via JavaScript, of the parameters required for the dilution into the PrepWorks protocol template. This

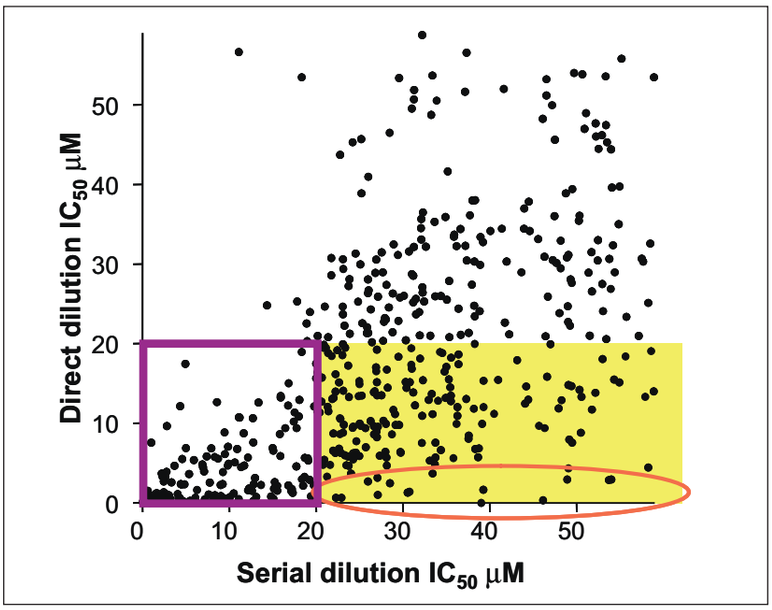


Figure 15: Comparison of IC_{50} values generated via direct dilution using acoustic droplet ejection (ADE) vs serial dilution. Researchers at BMS measured the IC_{50} values of more than 1,000 compounds over a six-log range of concentrations. The only difference between both sets of experiments was that the different concentrations were prepared either by direct dilution with ADE or serial dilution. The horizontal axis shows the IC_{50} value determined via serial dilution while the vertical axis gives the value determined via direct dilution. Compounds that had IC_{50} values greater than 20mM were considered ineffective. Compounds in the lower left, highlighted by a purple square, are hits by both techniques. The 110 compounds (>10% of total) in the yellow area were measured as ineffective by the traditional serial dilution technique but were active with direct dilution of ADE transferred volumes. The compounds circled in red are very potent candidates that had been falsely identified as inactive when the dose-response curves were generated via serial dilution. The true IC_{50} values of some of these compounds were two orders of magnitude lower than suggested by serial dilution. Recently, a second major pharmaceutical company confirmed these trends. They showed, using mass spectrometric analysis, that many hydrophobic compounds are lost during serial dilutions and that the actual concentration of the compound may be 10 to 100 times lower than expected. *Figure modified with permission*[2]

Figure 16
The Labcyte POD™ 810 plate assembler is optimised for direct dilution by acoustic drop ejection

Figure 17
An integrated liquid handling cell on an RTS Life Science robot with high throughput serial dilution capability

functionality enabled individual dilutions to be performed on a plate by plate basis, as part of a fully automated process under the control of d-Sprint™. Features such as multiple copies, different concentrations and custom dilution patterns can all be specified. In addition to the VPreps, the system includes two Thermo Cytomat storage units (one with assisted thawing capability), a plate sealer and piercer from Abgene, RTS' own-design 384 tube sealer/cutter instrument, as well as numerous lid handlers and barcode readers etc, all of which are controlled via RTS' Sprint™ scheduling software. Directly fed from an RTS SampleStore™, itself running d-Sprint™, via the integrated high-speed 384 Multi-Pix™ picking station, this system provides users with highly functional serial dilution capabilities capable of operating 24/7 (**Figure 17**).

Figure 18
TTP LabTech's mosquito® nanolitre pipettor uses disposable positive displacement microplates for compound dilution

Siliflow (www.siliflow.com) designs, manufactures and integrates liquid handling solutions. Its proprietary Nanodrop module is based on a piezo-actuated dispenser which allows ejection of droplets in the nanolitre to picolitre range (50pl to 10nl per drop). So far CVs of less than 3% have been achieved for nanolitre drops. Dispense frequency can be adjusted from 0.1 to 1Khz and exact number of drops (burst) can also be defined. The drop size is defined by the nozzle diameter while various electronic parameters allow to accurately adjust this drop size. Drop volume can be calculated thanks to a synchronised stroboscopic vision system combined with a proper calculation algorithm. Combining these items Siliflow aims to fully automate the direct dilution process. Siliflow will integrate these functions together with an agitation module, in a 3-axis robot in order to launch a commercial direct dilution system in mid-2007.

TTP LabTech's mosquito® product family (www.ttplabtech.com/mosquito) facilitates assay miniaturisation through precise compound dilution on a nL scale in microplates containing up to 1536 wells. At the core of mosquito is a continuous reel of disposable, positive displacement micropipettes which can aspirate, dispense and even mix. The narrow bore pipettes can sample from the bottom of each well, ensuring negligible dead volume. Since each micropipette is disposable, the instrument

guarantees zero cross-contamination of samples and removes the need for unreliable, time-consuming and expensive solvent wash cycles. This means compound dilutions can be produced directly from high-concentration DMSO stock solutions with confidence. Mosquito has two formats applicable to compound dilution: mosquito X1 is a single micropipette 'hit picking' version for precision sampling of any individual well; while the mosquito HTS uses 8 or 16 pipettes in a columnar format for more rapid pipetting. A key application of mosquito is the preparation of assay-ready plates within HTS departments. These plates contain test compounds prepared from library stocks ahead of time at the correct concentrations. DMSO is routinely used for compound dissolution, but its presence at concentrations above 1% can markedly affect assay performance. Thus, accurate nanolitre quantities of DMSO-based stock solution are required to keep concentrations low when preparing assay-ready plates. On mosquito, serial dilutions are commonly performed in a 500nL total volume using an aspirate, dispense and mix cycle. Where compound carry-over is an issue, tips can be changed for each dilution step. Alternatively, direct dilution can be achieved by pipetting stock solutions throughout the instrument's volumetric range. Routinely, this involves the repeat pipetting of 3-4 stock solutions at volumes between 50 and 1,200nL (**Figure 18**).

Standard serial dilution methods are tedious, time-consuming, and often inaccurate at high dilutions due to the propagation of errors. **Velocity11**'s (www.velocity11.com) Bravo™ Liquid Handling platform is capable of performing serial dilution by row or by column. Coupled with the VWorks™ software package, parameters such as mix height, tip retraction, mixing speed and mixing cycles can cut the time to perform an accurate and precise serial dilution by 75%. The software tools ensure that the serial dilution can be tailored to specific applications. The ability of one pipette head to perform both serial dilution and full 96- or 384- well dispensing, from low (2µL) high (200µL) volumes means that only one instrument is required to perform backfilling of plates and the serial dilution operation in a single protocol, with no head swap required. In a case study, we performed a standard 1:2 serial dilution by column across a 96 well plate. Using non-optimised parameters, 20 mix cycles were required to achieve satisfactory precision (average CVs under 3%, and accuracy >99%). The drawback was that this would require 20 minutes to process an individual 96-well plate. After utilising the capabilities of VWorks software platform to optimise parameter values for mix height, speed of mixing, and tip retraction, essentially identical accuracy and precision results with just three mix cycles was achieved; this reduced the time for a serial dilution operation to 5 minutes (**Figure 19**).

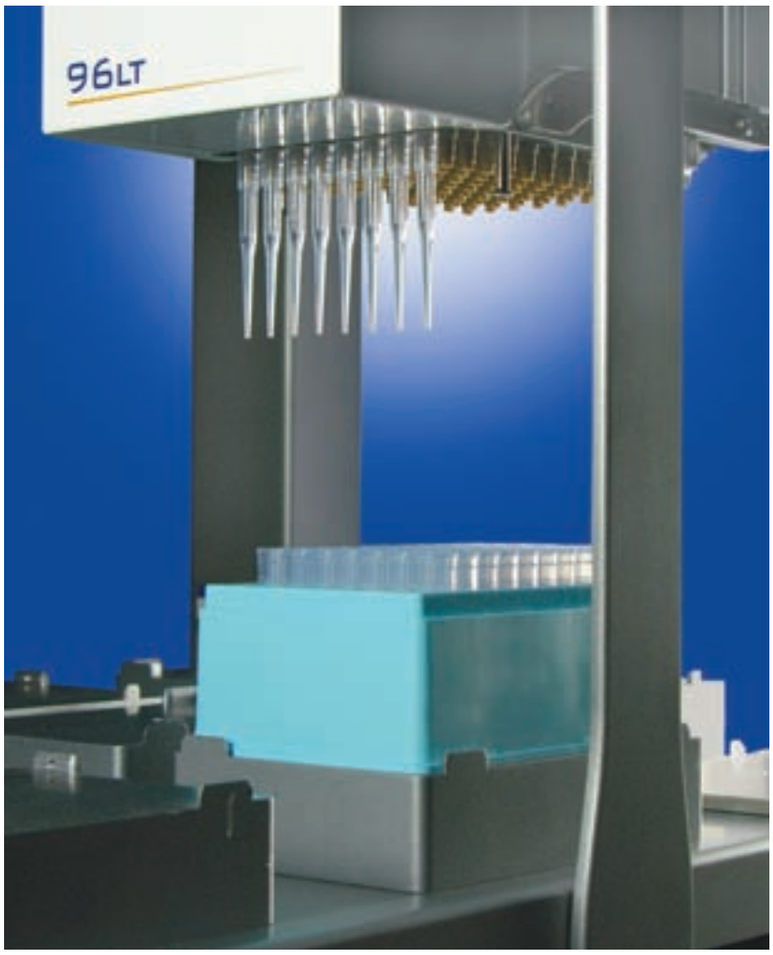


Figure 19
The Velocity11 Bravo Liquid Handling Platform. Tips can be loaded in single row or column and the full head mode

Summary

In **Table 1** we attempt to draw a comparison between the serial and direct dilution developments reviewed above. It also serves to highlight how direct dilution approaches can reduce or minimise the perceived inadequacies of current dose response analysis techniques. It is well recognised that even minor problems in the liquid handling or mixing technique will affect the dilution ratio and hence the compound concentration, and these errors will be compounded during the serial dilution process increasing with each successive dilution and transfer. It is, however, less appreciated that hydrophobic compounds may be readily lost

Table 1: Comparison of serial and direct dilution techniques

INADEQUACIES OF DOSE-RESPONSE ANALYSIS TECHNIQUE	SERIAL DILUTION		DIRECT DILUTION	
	DISPOSABLE PIPETTE TIPS	WASHABLE FIXED TIPS	USING ACOUSTIC DROPLET EJECTION (ADE)	USING OTHER FIXED NOZZLE NANOLITRE DISPENSERS
Accumulated error	YES	YES	NO	NO
Hydrophobic compounds lost	YES	not known	NO	not known
Potential for cross contamination	NO	HIGH	NO	HIGH
Wash waste	LOW	HIGH	NONE	HIGH
Consumables (tips) waste	HIGH	NONE	NONE	NONE
Source material required (volume)	µL	µL	nL	nL

from solution during aqueous serial dilutions with as much as 90-99% of the material absorbed to intermediate surfaces including disposable pipette tips and dilution wells. Direct dilution using ADE eliminates compound absorbance and adherence (sticking) since the sample does not encounter the pipette tips. It is not known the extent to which hydrophobic compounds might adhere to the inner surfaces of fixed tip pipettors or the nozzles of nanolitre dispensers, some of which may have specialised non-stick surface coatings. If disposable tips are used only one time, there is no chance of cross-contamination, although repeated use would increase the chance of carryover. As fixed tips pipettors or the nozzles of nanolitre dispensers come in contact with many different compounds on multiple occasions, even with the most stringent washing, there is the potential for cross contamination. To reduce carryover to an acceptable level it is routine to wash fixed tips or nozzles more thoroughly or frequently, and as a result the liquid waste stream generated will be high. If disposable tips are used only once, without washing, then the plastic waste stream also increases. By adopting a direct dilution strategy significantly less source material and less solvent are required to make sample concentrations than traditional techniques employing serial dilutions. Overall, the case for considering direct dilution would seem to be self-evident, particularly if by using ADE the quality of hits could be improved and their true potency revealed. However, it remains to be seen what value the groups responsible for making dose response comparisons will place on these benefits and whether direct dilution will be adopted further, particularly if this involves the purchase of new ADE liquid handler. DDW

References

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