1536-well assay plates: when do they make sense?

‘1536-well plates are still on the horizon; the low volume 384-well plate is for today.’

Tina K. Garyantes, Head of HTS, Bridgewater, Aventis Pharmaceuticals

There are three commonly cited reasons for miniaturizing HTS: (1) too many targets from genomics, (2) too many compounds from combinatorial chemistry, and (3) cost. Given finite economic resources, it is difficult today to fully exploit the large number of potential targets from genomics without overtaxing validation and post-HTS activities. Similarly, for economic and technical reasons, combinatorial chemistry could be best exploited for lead optimization rather than compound collection augmentation. Costs and less-commonly described factors, such as reagent or compound scarcity and logistic simplicity, do provide compelling reasons for miniaturization. The question then becomes, can these advantages be exploited at present or are there overwhelming disincentives to miniaturizing from 96- and 384-well plates to 1536?

Looking at cost factors first, the true cost of a typical 100 µl 96-well screen of half a million compounds is ~US$1,000,000. About half of this cost represents reagent costs (including compounds, and proteins or cells); overheads, personnel and consumables represent the other half. The screening cost per marketed drug is the cost per screen (e.g. US$1,000,000) multiplied by the number of targets that must be screened to produce one marketed drug. Assuming that 1 in 18 literature-derived screening targets succeeds, the screening cost per marketed pharmaceutical is US$18 million. This cost explodes if higher risk targets, such as unvalidated genomic derived targets, are screened. Assuming a 10-times lower success rate for such targets, the cost per commercialized drug becomes US$180 million. Clearly, this is unacceptable and must be reduced for affordable drug discovery.

Minimizing reagent usage

Because reagents represent the single largest cost, it is appealing to minimize reagent usage. Dropping assay volumes from 100 µl, which is typical in a 96-well assay, to 10 µl for a low volume 384-well assay generally drops reagent cost almost linearly with volume. The problem is that as assays are miniaturized further the actual reagent saving ceases to decrease linearly with volume because of the fixed dead volumes of existing instrumentation and reagent pricing schemes, which are ‘per well’ rather than ‘per volume used’. For instance, a typical reagent reservoir with a dead volume of 20 ml implies that, for a 10 µl addition of reagent per well and 100 plates per day, dead volume would account for ~5% of reagent usage. For the same 100 plates per day and 1 µl of reagent addition per well, dead volume represents 33% of reagent usage. And at 250 nl reagent addition per well, dead volume is a staggering 67%. Without more efficient pipetting systems further miniaturization ceases to provide savings below ~5 µl reagent addition per well.

The problem of waste

The next argument for miniaturization is compound savings. Some companies would like to augment their screening libraries with compound purchases of 1 mg. If used without waste at 10 µM, that 1 mg should suffice for the primary screening of ~2000 × 100 µl assays or ~20,000 × 10 µl assays. In most settings, screening even 2000 targets would support drug discovery for more than 10 years. The problem is waste. First, compound is left on the tips and plates. Second, aqueous dilutions can not be reused because of compound precipitation. However, if a year’s primary screening were performed efficiently with 1 µl of 10 mM compound (enough for 100 × 10 µl assays at 10 µM) and another 1 µl were used for follow up, 1 mg of compound would last 100 years. In other words, much more is gained by efficient compound use rather than miniaturization.

A stronger driver toward higher density plates is to improve screening efficiency and speed by having to move

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around fewer widgets per screen. Typically, the most difficult step in any assay is compound addition because each well has to be treated as unique. Unfortunately, because most current compound transfer devices are 384-well based, both the potential timing and the ease of use advantages of 1536-wells are limited.

Why not miniaturize?

There are also reasons not to miniaturize and push to 1536 well plates. First, there is substantial capital investment required for laboratories that are not already outfitted with pipetters and automated systems capable of reliably addressing the current 1536-well plates. Today's plates typically require precisely maintained CyBiWells™, Platemates™ or Platetracks™. In addition, until the Aquamax™ and, potentially, the Asys™ dispensers were available, only the Pixsys™ was available for bulk reagent dispensing that is routinely done with a Multidrop™ into 384-well plates. For reading, 1536-well compatible readers tend to be more expensive then those limited to 384 wells, and the speed advantage of 1536 is difficult to obtain without expensive charge-coupled device (CCD camera) detectors.

A second difficulty with 1536-well plates is that the assay variability tends to be greater in 1536 versus low volume 384-well plates. This increased variability tends to extend assay optimization. Because primary screens are typically only a few weeks long, any increase in assay development time is significant and argues against moving to 1536.

A third difficulty is 1536-well plates typically limit the type of assays that can be performed. For instance, the small wells are difficult to wash and high evaporation rates limit their use for cellular assays. In summary, the real drivers toward 1536-well plates and beyond are limited reagent availability and high reagent costs. Potential drivers to 1536-well plates and beyond are the promise of quicker, easier assays and compound savings. And the reasons to stay in low volume 384-well plates today are capital investment limitations, increased assay variability of the 1536-well format and limited reagent cost savings.

The future

Will this remain the situation over the next few years? No!

Low dead-volume, non-contact dispensers are now coming onto the market. Compound transfer technologies, such as pin plates, will enable the transfer of 1536 compounds simultaneously in neat DMSO from small compound reservoirs, improving both compound use efficiency and screening logistics. And new plate designs, such as 'virtual well plates', will solve pipetting difficulties and evaporation problems, enabling all existing formats, as well as some novel assay formats.

Virtual well plates are planar plates where 'wells' that hold the assay reagents are self-aligning hydrophilic spots in a hydrophobic field. One half of the well is created by the 'plate' and the other half is created by the 'lid'. Because of this configuration, many advantages are obtained, including the ability to easily perform capture and wash assays.

In addition, pipetting to virtual well plates is easy; little precision is required because the wells are large (1.5 mm diameter) and there are no sides to trap air. This feature enables 1536-well assays to run with virtually any pipetter on the market. Cellular assays are facilitated because of limited evaporation and a relatively large attachment area of >1 mm^2. Rapid kinetic assays can be performed because reactions can be started and stopped by simply closing the lid over the plate. In addition, mixing is rapid, the plate is extremely flat, assays can be split for multiplexing and other novel assay formats should be able to be incorporated.

At present, a 384-well low volume assay is an attractive alternative to a 1536-well assay. Almost all of the reagent, compound and cost savings that were promised by miniaturization can be obtained with no increased investment for either capital or miniaturization expertise. In the future, the ability to transfer all 1536 compounds in a single movement, the ease of use and flexibility of new plate designs, such as virtual well plates, and the reduced dead volume of reagent dispensers for bulk reagent dispensing should overcome most of the current barriers to 1536-well plates. Therefore, 1536 will probably be a format of the future, although not the only one. The success of HTS will primarily be determined by the wise application of the correct assay and robotic technology to the biology, rather than fitting the biology to the technology at hand. 1536-well plates are just one tool in the arsenal of successful screening groups.

Tina K. Garyantes
Head of HTS, Bridgewater
Aventis Pharmaceuticals
JR1-3201
Route 202/206
Bridgewater, NJ 08807, USA
e-mail: Tina.Garyantes@aventis.com