



Evolution of cell-based reagent provision

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Cell-based screening is now part of all stages of drug discovery, and therefore, cell supply is a rate-limiting step. Reagent provision groups have responded by exploiting automation and new concepts such as frozen cells to ease the constraint and increase quality and flexibility of cell supply. With increasing numbers of projects to support, reagent development is now perceived as a new bottleneck. In this review, we describe a new operating model that has emerged at Pfizer UK addressing reagent development and cell supply issues without growing headcount, by complementing the application of internal expertise with use of contract research organisations.

Primary plate-based screening in drug discovery searches for chemical structures that will serve as the starting points for drug development. Early in this decade, such screening was achieved using biochemical assays, exemplified by radioligand binding assays performed in 96-well microplates. Technology development has enabled modern primary screening to utilise live cells for greater than 50% of high-throughput screening (HTS) [1–3]. The significant benefit of screening using live cell assays is the generation of more physiologically relevant data and the ability to elucidate compound effect on target biomolecule function. As pharmaceutical compound collections have expanded from 10^5 to $>10^6$ compounds, miniaturisation of assays to 384- and 1536-well microplates have increased daily screening throughput beyond 10^5 data points [3–5]. These changes have compelled reagent provision groups to provide increased numbers of live cells on a daily basis. Previous HTS required the advance provision of biochemical reagents, such as membrane preparations, which could be met by a small team of manual cell-culture scientists. In contrast, a typical cell-based HTS at Pfizer (Sandwich, UK) now uses 1×10^{10} plated cells (excluding maintenance of cell stocks and requirements for assay development) and one full-time employee (FTE) is only able to support two HTS campaigns at any one time by manual cell culture.

Advances in cell-based screening technologies are not the only factor that has increased the demand for live cells. For example in

many pharmaceutical companies, plate-based screening within therapeutic areas during lead optimisation has been replaced by a centralised screening paradigm. A centralised set up is expected to decrease cost and expenditure of time by focussing expertise, minimising duplication and facilitating the application of standardised best practice. However, centralised screening groups have new requirements with respect to both quantity and quality of cell-based reagents to perform with the expected efficiency.

As these factors have increased the demand for live cells throughout the entire drug discovery process, manual just-in-time cell supply has become an inefficient strategy for cell provision. This approach is prone to frequent failure and is often not compatible with the consistency of cell provision required for assay success [6,7]. Consequently, cell provision became a limiting factor for both throughput and quality of cell-based screening [8]. Challenged with the concurrent demand for more cell lines at a larger scale, reagent provision groups have responded in three distinct ways; firstly by improving the robustness of the just-in-time cell supply process through automation and secondly exploiting frozen cell methodology to decouple cell production from screening. Thirdly, these approaches have been combined with outsourcing to increase overall capacity.

Addressing the cell supply bottleneck

Cell supply using automation

Cell-culture automation became a reality in 2001 with the launch of SelectTTM from The Automation Partnership (TAP) which was

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joined by similar systems such as AcCellerator™ from RTS Life Science International, Cell^{host} from Hamilton and more recently MACCS™ from MatriCal. The initial capital outlay for automated cell culture is high and therefore is most frequently applied within the pharmaceutical industry for the provision of cells to HTS platforms. The cell-provision bottleneck in a smaller biotech setting or for lower-throughput screening could be eliminated with a more cost-effective solution. In automating plated cell production, the actual process is largely unchanged but performed with far greater efficiency. For example, cell-culture automation at Pfizer (Sandwich, UK) enables six cell-based HTS campaigns to be resourced in parallel by an automated system and one FTE operator; for manual provision, three FTEs would be required for the same throughput. In addition to freeing up FTEs from routine cell culture and facilitating their redeployment to higher value-added science, an automated system guarantees that each cell plate is prepared using an identical protocol and has the ability to work 24 hours/day, 7 days/week with minimal operator input.

Most cell lines are amenable to automation and assay robustness is improved when compared to manual provision of plated cells [6,8,9]. Figure 1 shows an example of the impact of automated cell culture within Pfizer's centralised UK screening group. This data tracks the quality of a challenging assay using a freshly plated neuroblastoma cell line. Before the introduction of automated cell culture, variable growth properties of this cell line resulted in frequent assay failure and as a consequence, extra cell supplies to enable additional assays per week were required as a contingency. Upon automation of the cell provision, there was an immediate and significant improvement of the assay robustness

and the efficiency of reagent provision, as the variability of cell-culture process and the routine preparation of cells for contingency assays was eliminated.

Cell supply using frozen cells

The utilisation of cryopreserved assay-ready cells (frozen cells) has revolutionised cell-based screening in recent years [7,10,11]. Intriguingly there are few publications describing the technique's best practices (e.g. freezing or resuscitation protocols) and rapid uptake has resulted in a lack of tailored, commercially available hardware to automate the process. The principle of frozen cells is simply to resuscitate and dispense batches of cryopreserved cells directly into assay plates without the need for ongoing cell culture. In contrast to the introduction of cell-culture automation, this concept is simple and inexpensive to test and implement in any screening environment, regardless of throughput or scale. Our own validation data has shown comparable pharmacology and signal-to-noise to fresh cells in almost every G-protein-coupled receptor assay; however ion channel screening has generally yielded higher quality data in fresh cell compared to frozen cell format (Cawkill *et al.*, unpublished). Once validated as a viable technique for cell provision, reproducibility is increased through use of a single batch of cells at the same passage for a prolonged period of screening. Frozen cells also increase efficiency within cell supply, both through reduced wastage as cells are only plated when required, and through the resource freed from routine maintenance of stock cells. Overall, this method has revolutionised cell-based screening through delivering the flexibility of a biochemical assay whilst continuing to produce the level of information and data expected of a functional assay.

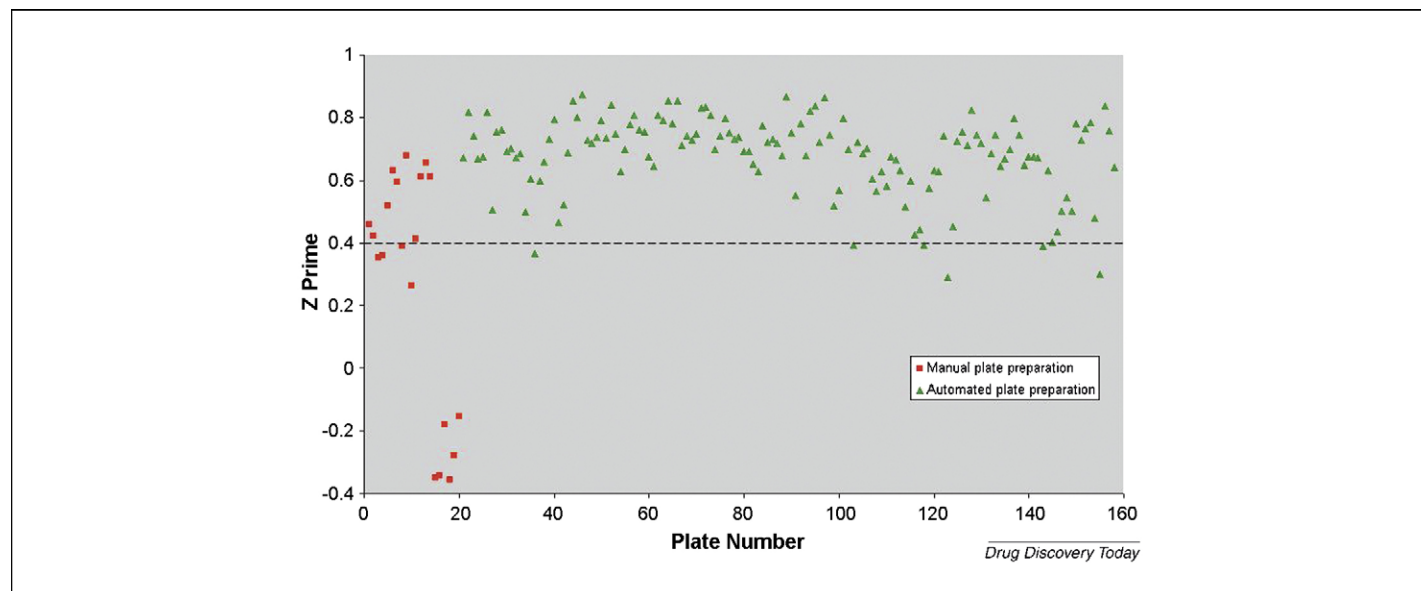


FIGURE 1

Data to exemplify the improvement in assay robustness when moving from manual to automated cell culture. The graph shows Z' data over a one-year time period for a sodium ion channel assay supplied with fresh cells. Transition from provision of manual plated cells (red squares) to Select™ plated cells (green triangles) occurred at plate 21. Defining assay failure as $Z' < 0.4$ (marked by a dashed line), then 50% of manual plated cells resulted in assay failure compared to only 4% of plates provided via Select™. The potency values of standard compounds were tracked during this timeframe and remained unaffected by the transition (data not shown). The switch to automated cell culture was made following validation data obtained from a series of manual versus automated comparative experiments. These experiments were performed by the same scientist in parallel to provision of cells for routine screening (data not shown). Although the precise reason for the severe failure of plates immediately before the switch is unknown, this highlights the advantage of reproducibility through automation since no severe plate failures occurred following the transition.

Outsourcing cell supply

Arguably, the most pressing demand on the future of cell-based reagent provision is to increase capacity and reduce the time to milestone decisions concerning reagent generation projects. As the demand to drive down both material and labour costs is at odds with the need to increase productivity and the capability to deliver increasing numbers of bespoke reagents in a timely manner, we must derive greater impact from technology and methodological advances. Following success in drug discovery chemistry [12], one emerging solution is the employment of contract research organisations (CRO) for the bulk production of cells. Several of the largest bioreagent suppliers have recently announced initiatives in the areas of reagent provision (e.g. GE Healthcare launching Cell Factory, Invitrogen acquiring CMT, Perkin-Elmer acquiring Euroscreen). An optimised protocol for production of frozen cells can readily be prosecuted at a CRO, making outsourcing such tasks a very effective and cheap enabler to project progression. As with the introduction of automation, outsourcing releases cell biologists from the routine aspects of cell culture to focus on other areas within the discipline.

In order to relieve a cell supply bottleneck, these three methods will work more efficiently in combination than in isolation. Constructing a cell-provision strategy to meet the requirements of a particular screening environment requires attention to the relative advantages and drawbacks of the methods outlined above (see Table 1).

The cell-provision strategy at Pfizer UK

Since adopting a centralised model for primary screening in 2006, cell supply at Pfizer (Sandwich, UK) has utilised an effective combination of automation, frozen cells and outsourcing to efficiently and effectively resource projects (see Figure 2). Our experience underlines that a combination of cell supply methodologies is needed to meet the differing challenges and priorities of screening during the different phases of drug discovery and that the efficiency of our strategy relies on projects changing their mode of cell provision as they progress. To avoid stalling projects during such transitions, we invest time during assay development to optimise reagent provision via both automated cell culture and frozen cells, along with the necessary protocols for outsourced cell production and QC.

HTS requires high cell numbers (typically 10^8 to 10^9 cells/day) over a period of weeks in order to screen high compound numbers (typically 10^4 to 10^5 compounds/day). A robust cell-provision process is also important but need only be sustained over a short time

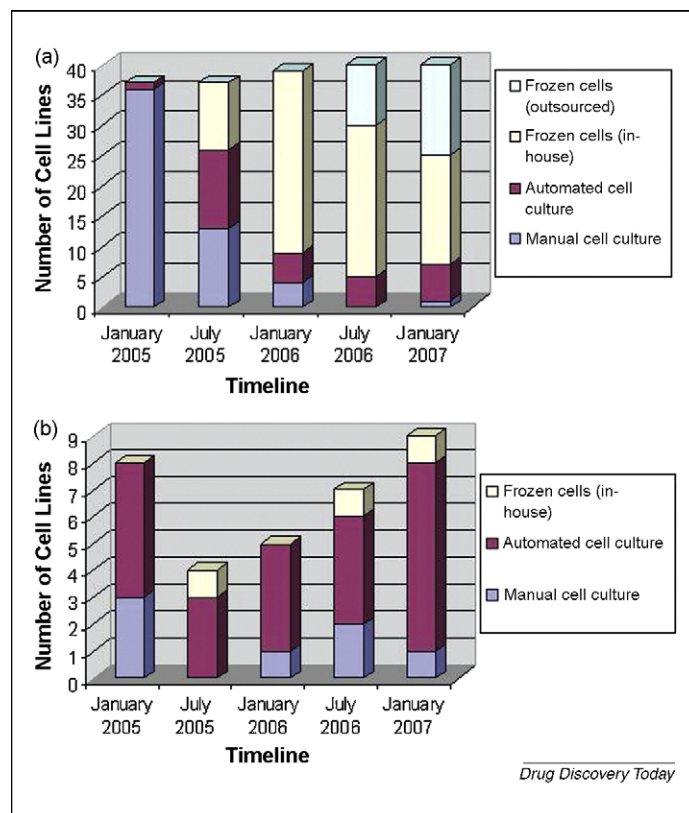


FIGURE 2

Evolution of cell supply method for primary screening at Pfizer UK (2005–2007). The data shows the method of cell supply used to provide cell-based reagents to both non-HTS primary screening (a) and HTS screening (b). Cell supply using frozen cells generated both in house and by contract research organisations has become the method of choice for non-HTS, post-hit optimisation screens, whereas automated cell culture has remained the most popular method for HTS cell supply.

period with assay throughput being the most crucial factor. These high volume but low capacity demands are most efficiently met through use of automated cell culture. An automated platform can quickly and efficiently meet the daily cell demands required, whereas a significant lead time and in the case of outsourcing, financial expense would be required to generate a frozen cell stock sufficient for an HTS campaign. Where automated cell culture is not available, frozen cells offer an alternative to continuous manual cell culture. Outside the big pharma, the lead time required to generate a frozen cell stock for HTS will be lower since compound collections are smaller. In contrast, screening in a lead optimisation scenario

TABLE 1

Comparison of the relative operating costs and practical issues for ongoing cell supply to screening using each method of cell supply

Implication	Manual cell culture	Automated cell culture	Frozen cells	Outsourcing
Capital investment	+	+++++	+	None
Ongoing expense costs	+	++	+	+++++
Ongoing FTE expenditure	+++++	++	+++	+
Barriers to implementation		Laboratory space specialist training	Not all projects are amenable to this method	Reliable protocol transfer
Benefits to implementation		Reproducibility, efficiency (output per FTE)	Capacity, flexibility (decoupling of screening and reagent provision)	Capacity (redeployment of FTE resource)

requires lower cell numbers (typically 10^6 to 10^8 cells/week) over a period of months to years but with an increased capacity to supply up to 50 cell lines in parallel. Compound numbers are lower (10s to 100s compounds/week) but rapid turnaround of data and flexibility to change screening plans are more crucial than throughput. These low volume but high capacity demands can be efficiently resourced through production of frozen cell batches and this methodology has emerged as the primary mode of cell supply. During lead optimisation, a single batch of validated frozen cells can resource months of screening without the need for further cell culture. The lead time to generate such batches is not detrimental to project progression and the expense of outsourcing is justified by the impact on long-term project support. In our experience, partnering with vendors for the routine production of established frozen cell reagents has proven effective and allowed us the strategic flexibility to redeploy internal resource to focus upon particularly challenging reagent provision projects and the improvement of our own technology capabilities and scientific practices.

Cell-provision methods can have a direct impact on assay performance and quality: analysis of the Pfizer UK 2006 cell-based screens during lead optimisation highlights a reduced failure rate for assays supplied with frozen cells (11%) compared to manually prepared cells (17%). However, the lowest failure rate was seen for automated cell provision (6%), reflecting the greater consistency achieved by automation. The lack of reproducibility that manifests itself in manual cell provision [13] is replicated, albeit to a lesser degree, when working with frozen cells and reflects that the method still contains a significant element of manual cell culture. The reduction in failure rate compared to manual cell culture does illustrate the clear benefit of frozen cell aliquots together with the flexibility afforded by decoupling reagent provision and assay prosecution.

Cell line development – a challenge for reagent provision

Bespoke recombinant stable mammalian cell lines are paramount for the successful screening of a large proportion of drug targets [13–15]. If a project successfully progresses all the way to the clinic, then the reagent is used over several years and will cause significant delays to project progression if associated with a high assay failure rate. Typically, a suboptimal reagent results from insufficient characterisation at the project outset due to the pressure of reducing idea-to-screen times for exploratory projects. However, techniques for the generation of recombinant cell lines have seen less development than those improvements described for cell supply. Consequently, development times for these reagents remain unaltered and projects must endure months of lead time before screening [16]. Project progression to lead generation and beyond also requires a complex screening cascade with different assays and cell lines [7]. Functional and binding assays are employed simultaneously to understand the pharmacology of a compound and in addition, species orthologues are profiled for translational biology information. With the increased demand for cell lines per project and the lack of significant new time-saving technology, reagent development is now the bottleneck within cell provision. Two concepts can be applied to cell line development to alleviate this problem. Firstly, automation has the potential to improve both quality and quantity of a well-established but

manual process, such as stable cell line development; secondly, transient transfection is an alternative methodology to work in combination with the well-established stable cell line construction process.

Improving the generation of cell-based reagents

Implementation of automation into stable cell line development

The basic workflow for generating a stable cell line comprises three stages: (1) transfection and selection of a heterogeneous pool of cells, (2) isolating cell clones and screening for suitability of use and (3) clone characterisation in the assay format(s) of choice [16,17]. The last significant advance in stable cell line generation for drug discovery was the introduction of fluorescence-activated cell sorting and reporter gene technology to identify cells with a suitable response in a functional assay at the second stage of this process [17–19]. Stages 1 and 3 have remained without noticeable change or application of technology or automation despite the capability of cell-culture robotics to perform transfection, cell maintenance and plating, leaving the entire process typically requiring three months for the generation of a well-characterised clonal cell line [16,20]. Early use of cell-culture automation during cell line development, particularly for the characterisation of promising clones, would improve efficiency by merging the reagent and assay development stages, reducing the overall time required to establish an assay by several weeks. This should be increasingly feasible as the use of frozen cells frees up capacity in cell-culture automation.

Recent literature reports the continued improvement of stage 2 technology (facilitating the generation and screening of clonal populations) in the development of antibody producing cell lines [21]. The principle reason for focussing on this stage is that these experiments are historically crude in design since the volume of clones to be processed is high and frequently limited by the endurance of the scientist. Thorough analysis of hundreds of clones is desirable but rapid elimination of clones is necessary to reduce workload to a manageable level. New automation technology (such as Cello™ from TAP) greatly increases the capacity of stable cell line development within a given timeframe. However, capital costs for these instruments are high and the overall timeline for enabling idea to screening will remain almost unchanged. These factors mean that automation is only a practical solution in a setting where the volume of stable cell lines required is constantly high.

Exploitation of transiently transfected cells

Transient transfection as a means of generating cell-based reagents has always been an option for any part of a screening campaign. However, until recently this method was only suitable for generating just-in-time target expression and not sufficiently robust or logistically sustainable over a number of years for a long-term screening campaign. The emergence of frozen cell methodology and subsequent decoupling of the transfection from assay execution has enabled the use of transient expression systems for the rapid construction of cell-based screening reagents [13,22], potentially reducing the lead time from months to weeks. While some reports describe the use of transient expression for large-scale screening [20,22], there are explanations for the lack of widespread use. Main reasons include the uncertainty of the pharmacology

derived from a heterogeneous population of cells [12], the quantity of DNA required and the cost of transfection reagents, as well as the potential for batch-to-batch variation. A solution to some of these problems is viral transduction. Recent publications report that baculovirus can deliver and transiently express a range of target receptors in mammalian cells yielding excellent screening data in a far shorter timeframe [23–25]. Other advantages of a viral transient system are the controllable and tailored expression level, ease of virus scale up and the elimination of expensive transfection reagents, making it a very attractive methodology for reagent provision.

Considering the generation of new cell-based reagents at Pfizer UK, we now aim to focus on transiently expressing cell lines for exploratory efforts. This allows the project rapid access to a functional reagent, reducing idea to screening timelines by several months and therefore accelerating decisions regarding project progression. As confidence in both project rationale and the screening cascade increases, automation will be utilised further for the smooth transfer of a stable cell line from generation (e.g. Cello™) to optimised growth and scale up (e.g. SelecT™). The need for reproducibility within screening means that transiently transfected cells are less appropriate at the later stages (e.g. lead optimisation). However, infrequent ancillary screens (e.g. species orthologue profiling) are well supported by a single batch of frozen transient cell-based reagent which can last the lifetime of the project.

Further developments and trends

In recent years, the changing demands of the screening environment have necessitated an evolution in the way cell-based reagents are generated and supplied. Clearly there is still potential for improved automation solutions, for example, the development of an integrated system for the preparation of frozen cell aliquots. However, it is likely that the biggest gain for future reagent provision efforts will not come from automation alone but from applying a combination of strategies to best exploit the advances

in reagent generation and supply as described herein (see Figure 3). At the early (exploratory) and later (beyond lead optimisation) stages of projects, assigning and exploiting the clear benefits of each approach is straightforward. It is far more difficult to generalise at the stage when projects transition through assay development and into a routine screening cycle.

Cells are not the mystical entity that they might have been traditionally thought as demonstrated by recent cell methodology advances and treatments previously regarded as implausible. For example, pausing cell growth by reducing the incubation temperature has been described [26] and could bring further flexibility to the just-in-time cell supply process. Cryopreservation of cells growing in flasks has also been reported, which has the potential to reduce the time needed to recover and expand fresh cells [27]. Taking this a step further, cryopreservation of cells directly in assay plates would enable an automated cell-culture system to produce plates for freezing, therefore combining the benefits of both automation and frozen cells. However, frozen storage of large plate numbers needs to be addressed before this strategy can be implemented as routine.

The emergence of automated platforms for technologies such as high-content screening [28] do not necessarily represent the beginning of a marked shift in primary screening strategies, although they indicate the requirement for delivery of bespoke cell lines for such screening approaches. In contrast, one could argue that the current development of label-free assays may see an end for the need to achieve recombinant expression of receptors. The use of primary cell lines or indeed stem cell lines for routine primary screening shows promise but has yet to become established [29,30]. Technologies such as CellKey™ (MDS Sciex) and RT-CES™ (Acea Biosciences) exploit endogenous receptor expression to measure activity of targets [31,32]. However, these technologies do not currently have an impact on primary screening as they are limited to 96-well format and many other emerging assay formats such as Tango™ still require recombinant expression of receptor (see <http://www.sentigen.com/technology/tango.php>).

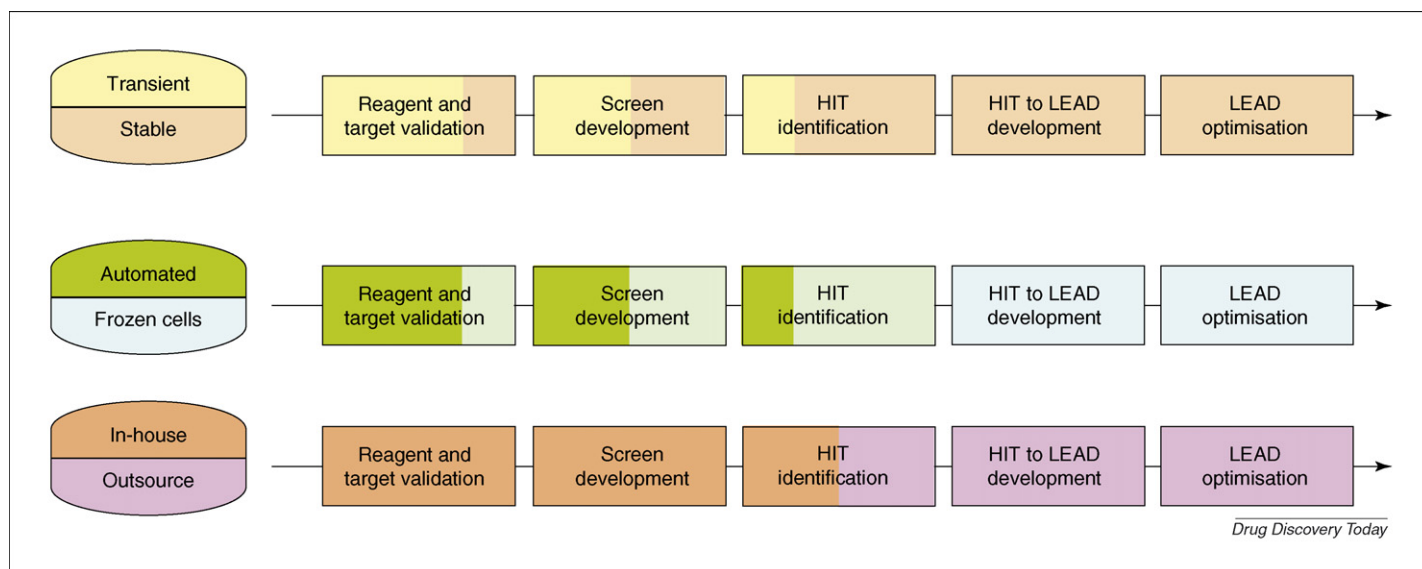


FIGURE 3

A desired distribution for the near future of reagent provision within the pharmaceutical industry. The chart demonstrates an optimal combination of strategies that would enable robust and timely cell production for primary screening in the pharmaceutical industry.

Conclusion

The increased use of cell-based assays for screening has rapidly driven developments and new concepts in reagent provision. Automation has enabled the scaling up of established processes with improved quality and reduced resource, driving a 24/7 operation in reagent provision and screening teams. Frozen cells have transformed the logistics of cell-based assays and have put them on par with biochemical assays with respect to the just-in-time preparation of reagents and the validation of large reagent batches. Transient expression systems add the option to support a large number of exploratory projects to sustain the downstream project pipeline without the resource intensity of traditional approaches focussed on cell lines, that is, they minimise the 'investment at risk' in reagent development and provision teams. For building a coherent strategy in your organisation, there is no single 'right'

solution to the cost-effective and timely supply to projects at different stages of the discovery process. Dependent on the size of the organisation – small biotech versus large pharmaceutical – and the lifecycle of the individual project, reagent provision strategies have to be tailored using a combination of the methods outlined. Rather than large investments into single technologies, it is important to adopt a flexible strategy using the internal expertise and resource as well as exploiting the opportunities presented by contract resource organisations.

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