Future considerations in HTS: the acute effect of chronic dilemmas

Matthew A. Sills

Today, in the area of Biomolecular Screening, it would seem that 'something better' in terms of instrumentation or assay technology, is emerging almost every week. From the standpoint of planning, this presents significant challenges to decide whether to integrate the 'latest and greatest' advance, whether to wait just a few months for the next advance, which may make the current advance obsolete, or perhaps to maintain the status quo. This decision would be considerably simpler if there was a clear, optimal strategy for identifying and selecting lead compounds. This article attempts to look at this issue from the perspective of discussing the pros and cons to several approaches to lead discovery.

It happens every time I return from one of those 'screening' meetings, I just can't get to sleep. Too many questions running around inside my head.

- When should we implement screening in 1,536-well plates?
- What about the 9,600 format?
- Will these formats become obsolete with 'assay-on-chip' technology?
- What instrumentation should we invest in?
- Which of the new assay technologies should we integrate?

Maybe one of these years, there will be a six-month period where we can just 'run the program' as it is, rather than continually having to evaluate and determine what equipment or technology should be rapidly integrated into the biomolecular screening program because of the new advances that are constantly being made in this area. Questions, questions, questions; how to decide, decide, dec... [fade into semi-consciousness].

CC: Colleagues, welcome to tonight's debate between left brain and right brain. I'm C. Callosum and will be moderating our discussion on 'where should HTS be heading in the future?' Tonight, left brain (LB) will present the view that 'more is better'. The challenger, as usual, is right brain (RB), and will be presenting the opposing viewpoint, 'is all this really necessary?' Just to remind you, we need to maintain civility. We must avoid a recurrence of what transpired during last week's debate on 'Si Dyslexia Reayll a LemProb in DayTo's Socieyt?' You must refrain from using phrases such as 'lame brain', 'oh dominant one' and 'Alzheimer breath'. You'll be penalized one neuron for each infraction, where apoptosis will be induced by induction of protease activity [gasp from audience].

Let's begin the discussion. LB, would you like to start?

Mission and program capacity

LB: In today's world of biomolecular screening, there is overwhelming evidence that HTS is an invaluable tool in the drug discovery process. Not only are lead compounds being identified –

Matthew A. Sills, Novartis Pharmaceuticals Corporation, 556 Morris Ave., LSB 1147, Summit, NJ 07901, USA. tel: +1 908 277 7292, fax: +1 908 277 2084, e-mail: matthew.sills@pharma.novartis.com

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CC: LB, can I interrupt you a minute, and ask you to define the term, lead compound.

LB: We consider leads as those compounds identified in HTS where any chemical optimization efforts have been initiated to improve activity, selectivity or reduce toxicity. As I was saying, not only are lead compounds being identified from HTS, but compounds derived from these leads are now in development; some have even made it to the market, such as Boehringer Ingelheim's reverse transcriptase inhibitor, nevirapine (Carol Homon, pers. commun.).

RB: That's fine, so why aren't these efforts sufficient? It would seem obvious based on these successes that we should continue at the current level of resourcing. I sense, like usual, you're trying to tell us we need to do more.

LB: That's correct. The goal of screening has changed dramatically in the past ten years. You remember in the late 1980s, where we had to grovel just to get an assay to run in the screening program, or is your Alzheimer's getting worse?

CC: All right. Enough of that. You've just been penalized one neuron [a scream of a dying neuron can be heard in the background]. Now, let's keep to the issues, shall we?

LB: All right, all right. The point is, in the late 1980s, although HTS programs existed, HTS was usually not looked upon as an accepted means of providing lead compounds. Research projects were initiated, and maybe after three to four years of exploratory work, the projects were challenged to identify lead compounds. So instead of just trying to identify hits, which we define as compounds with activity in a specific assay, the goal evolved into identifying lead compounds. Although the present objectives of HTS are adequate, they do not go far enough. It's not sufficient to identify compounds with activity in a particular assay that are attractive to chemists based on structure or potency alone. There is now a need to find 'quality' leads, leads that are selective for the particular target, leads that are bioavailable, leads that, in most areas, lack toxicity.

RB: I agree that we need quality leads. But with the dozens of hits being identified in HTS, the project teams should bear most of the responsibility of selecting quality leads and developing them into clinical candidates. We should not have to waste our time screening more compounds.

LB: I disagree with you on that point. Today, many companies are evaluating upwards of 100,000–300,000 compounds per assay. With a hit rate of 0.1%, 100–300 hits are identified. Just from the perspective of stability, structure and potency, perhaps 30–50% of the compounds will not be of interest to the project team, with the result that perhaps 1–2 or, if you're fortunate, three or more lead series are identified. However, if we take into account compound selectivity, toxicity, bioavailability or effect on drug metabolism, those lead series may rapidly drop out of consideration. Or, it may take many months before these properties can be effectively designed into the molecules, in which case we've moved the bottleneck in the drug discovery process from lead identification to lead selection. What we need to provide, then, are more hits that can be profiled (not necessarily in the lead finding area) in selectivity, toxicity and cell permeability assays in order to deliver 2–5 'quality' lead series that have these properties. To do this, we need to increase the number of compounds screened per assay.

RB: And how many hits and compounds did you have in mind?

LB: To identify 2–5 quality lead series, we probably need to identify about 500–1,000 hits, so we'll need to screen somewhere around 500,000 compounds, maybe even 1,000,000 compounds per assay within a timescale of a few months.

What about compound diversity?

RB: Are you out of your half-a-mind! Why is it that your answer is always that we have to be doing more, rather than being more efficient? What we should be focusing our efforts on is generating a library of 100,000 compounds with optimized diversity, rather than your approach to screen every compound in existence. Have you completed that diversity analysis we talked about in last April's dream?

LB: Not yet. The longer we've worked on the problem 'di-verse' it gets [pause for groaning] (from S. Adams, Dilbert cartoon). Seriously though, screening an optimized library of 100,000 compounds may make sense, but how do you know that you can adequately represent sufficient diversity in a library of only 100,000 compounds?

RB: If adequate software tools are developed, and it's just a matter of time before this will occur, an optimized library of 100,000 will be sufficient. If one lead can be obtained from screening a non-optimized library of 100,000 compounds, then why shouldn't five to ten or more leads be obtained from an optimized library.

LB: Perhaps, but there are too many examples where only a small change in structure produces a tremendous change in activity. I'm not just talking about changing a methyl to an ethyl substituent. What about our experience from that kinase assay where only one of a series of analogs was active? Unless that one compound would have been selected for an optimized library, which is highly unlikely, a development candidate would have been missed.

RB: Well... you may have a point there. But let's say for a minute that 1,000,000 compounds per assay is a target to aim for, which I'm not conceding. Where does this end, at 10 million compounds per assay, 100 million compounds per assay?

LB: I wish I knew. Look, five to seven years ago, most of us would have thought that the concept of needing to screen 100,000 or even 200,000 compounds per assay was crazy. Today, very few would challenge this concept. I'm not saying that it's necessary to screen an entire compound library in every assay. And perhaps a small, optimized library, based on results from previous assays from a similar target class, will be sufficient for certain targets, such as kinases, proteases or G-protein coupled receptors. But the number of compounds screened needs to increase if we are to find quality leads.

And that's not all that needs to increase. There's another issue with respect to capacity, and that's the number of assays needed to be evaluated per year. Financial analysts have indicated that the larger pharmaceutical companies will need to bring two to three, or even more compounds to market each year in order to maintain double-digit profits in the future. To get, let's say, two compounds on the market each year, we're going to need about 12–15 projects delivering compounds to development. Since every project is not successful, and not every assay generates leads, we need to run 120–150 assays per year.

RB: Whoa there, fella. I see those dollar signs in your eye again. I know there supposedly is a 10% success rate from screen to development (R. Spencer, from oral presentation at IBC Conference, *Integrating R&D Activities to Accelerate Drug Development*, 11–14 August 1997, San Diego, CA, USA), but we must improve the efficiency of lead finding; for example, by screening better targets. Secondly, I know you think the world revolves around HTS, but there are other avenues to identify leads, such as rational drug design. So, let's say for a moment that 140 assays are required to obtain leads for 14 programs to get two marketed products annually. If 50% of the programs identify development compounds from other sources, then only 70 assays per year are required. If we increase the efficiency from 10% to 20%, we're now at 35 assays per year, which is a somewhat more manageable figure.

LB: You can't just snap your dendrites and expect to double the success rate of lead compounds going into development. There is no precedence to show that this can, or will, occur.

RB: Have you been comatose for the past few years? I think it's pretty clear that functional genomics will increase the success rate by providing better, validated targets.

LB: Although I'm sure this will occur in the future, I have yet to see the deluge of better assays that were to fill the 'gene to screen' pipeline. So, I'm somewhat skeptical about the short-term impact of functional genomics on lead compounds.

CC: Gentlemen, can we leave that subject to next month's scheduled dream scenario and get back to discussing the issue of screening 1,000,000 compounds per assay?

RB: All right. Do you realize what kind of resources will be required to evaluate that number of compounds? Maybe we should refer to you as EB, rather than LB; not for empty brain (although if the hat fits, wear it), but for empire builder.

Miniaturization

LB: I grant you that more resources will be needed. You can't get increased productivity without some increase in resourcing, although recent down-sizing trends have attempted to refute this idea, albeit unsuccessfully. That's why we need to utilize miniaturization and new technologies to obtain a multifold increase in productivity. This will enable us to generate a five- to tenfold increase in productivity for a smaller investment in resources, such as 30–50%.

RB: There you go again with miniaturization. If I had a neuron for every time I've listened to your claims

Table 1. Impact of miniaturization on reagent cost

Plate	Number of compounds per plate	Number of plates	Volume of protein added	Assay volume	Total volume of protein added[a]	Cost
96-well	88	2,273	10 µl	100 µl	2,182 ml	$436,400
384-well	352	568	2 µl	20 µl	436 ml	$87,200
1,536-well	1,408	142	0.4 µl	4 µl	87 ml	$17,400
9,600-well	8,800	23	0.02 µl	0.2 µl	4.4 ml	$880

[a]Product of (number of wells; col. 1) × (reagent volume; col. 4) × (number of plates; col. 3).

about how miniaturization will solve all of our HTS problems, we'd have an IQ over 1,000! Tell me, with the multiple types of formats emerging, such as 384-, 864-, 1,536-, 3,456-, 6,144- and 9,600-well plates, what format should we be moving to?

LB: 384 and 1,536, and possibly 9,600 or 6,144.

CC: Why those numbers, LB?

LB: Most of them represent a linear fourfold expansion in the 96-well plate (i.e. 96, 384, 1,536, 6,144). The 9,600-well plate would also seem to merit consideration at this time, based on the demonstrated feasibility of using these plates[1].

RB: Gee, only four formats. Do you realize what the cost will be to integrate this many formats? Have you given any thought to the problem of how utilizing multiple formats will impact fully automated robotic systems?

LB: Although it will be a challenge to adapt robotics to multiple formats, I think this will occur with time. But more importantly, I think the more relevant question is what will be the cost if we don't miniaturize? Let me give you an example of the added value in miniaturization. In this example, let's say we want to evaluate 200,000 compounds in an assay where the cost of the protein is $200,000 for one liter of material. If we use 96-well plates, where one compound is evaluated per well in singlicate determinations –

RB: You screen in singlicate!!!

CC: Hello, RB. Are you there? It seems RB has just fainted. Increasing blood supply to RB... He's coming round...

RB: ... Where?... What?...

CC: We'll hold discussion on that topic until later. For now, let's focus on miniaturization. LB, please continue.

LB: As I was saying, if we use 96-well plates, with 88 compounds per plate (the other wells are for controls), 2,273 plates will be required to screen 200,000 compounds. If the volume of protein added is 10% of the assay volume, which is 100 µl, then the total volume of protein will be 2,182 ml at a cost of $436,400 (Table 1). For the other plate formats, we'll make the assumption that compounds are dispersed as multiples of 88 compounds per plate. You could probably fit more compounds onto the plate, but let's use this worst-case scenario.

Using similar calculations for the other formats, you can see that phenomenal cost savings can be realized through miniaturization, where the cost of protein in this example can be decreased from $436,400 to $880! The cost of the protein is reduced more than 100-fold because there is a 500-fold reduction in assay volume. I think even you would find it difficult to argue with these numbers.

To what extent will assays need to be miniaturized?

RB: Okay, I agree... but only to a certain extent. Let's look at your data for a minute (Table 1). I agree there is a compelling argument to miniaturize. But I think we have to look at the issue of added value. For example, I think it is clear from the data that there is significant added value in reducing the assay to 384-well plates, where a saving of $349,200 can be realized. I would even concede that there is significant value in reducing the assay to a 1,536-well format, where a further saving of $69,800 can be realized. But, I have a major issue with further miniaturization, be it with 9,600- or 6,144-well plates.

Due to the submicroliter volumes, there are more inherent problems in running assays in 9,600- than 1,536-well plates, such as edge effects, evaporation and static electricity. If we are to screen compounds in duplicate or triplicate determinations using 9,600-well plates[1], and we can't use the edge of the plate, we're

now talking about a throughput of ~3,000 compounds per plate. The question that needs to be asked at this point is whether the trade-off in quality and reproducibility in running the assay in a volume of 0.2 μl vs 2.0 μl (for 1,536-well plates) justifies further miniaturization. Along these lines, is there really significant value in screening in 9,600-well plates when there is only a cost saving of $16,500? Is this really an unreasonable cost for evaluating 200,000 compounds?

LB: I see your point. So what would you suggest concerning miniaturization?

RB: I'm not sure. I do agree, based on your argument, that we need to miniaturize to balance capacity and cost issues, and perhaps 1,536-well plates are a good target to aim for. But don't you think 384-well plates will be a transient format, particularly with the advent of 1,536-well plates?

LB: No, not really. Just as we will probably have to run some assays in 96-well plates for the foreseeable future, I believe that 384-well plate assays will also be maintained for some time because we may need to maintain a certain volume to obtain an adequate signal.

RB: But aren't you concerned that the cost of purchasing instrumentation to detect the signal in all of these formats will be prohibitive?

LB: In what sense?

RB: In the sense that you will need instruments for each type of plate format to detect, let's say, fluorescence, in 96-, 384-, 1,536- and 9,600-well plates.

LB: That decaf coffee must be getting to you again. Where have you been during the past two years, with the emergence of digital imaging? There are imaging systems that are available today that can easily detect fluorescent and luminescent signals in many types of miniaturized formats.

RB: When you're left, you're left; digital imaging is having an impact but, I wouldn't describe these systems as 'easy'.

LB: Why not?

RB: You really should get into the lab more often. What I mean is that imaging is not like current instrumentation where you load a stack of plates, push a button and in an hour or two you can obtain all of your data. Today, imaging requires significant work up-front to standardize the instrumentation before the plate is actually read, such as determining the optimal exposure time and correcting for uneven illumination across the microtiterplate. And, once the plate is read, significant effort is required to obtain data. For example, each image is contained in a separate file, which needs to be analyzed individually; it doesn't compare favorably with the stack of plates in your plate reader, which can be analyzed simultaneously.

And, one more thing. Whereas many of the instruments currently have stackers or are integrated with a robot, each plate currently has to be loaded manually.

Assay technologies

LB: These issues will be addressed. It will just take some time. Look, ten years ago, most screening occurred on receptor targets using filtration methods. At that time, we dreamed of a homogeneous methodology to eliminate the filtration step. Today, that dream has been realized with SPA (scintillation proximity assay; Amersham International)[2] and other proximity technologies, such as Scintiplates (Wallac Oy) or Flashplates (New England Nuclear). In the future, homogeneous technologies must play an even more significant role if we are to miniaturize assays successfully. And don't tell me, I suppose you think there is an issue with using homogeneous technologies, too?

RB: Absolutely.

LB: [muttered] I knew it.

RB: All right. What's your view on homogeneous technologies?

LB: These technologies will be essential for miniaturization and digital imaging. We won't be able to afford any separation or wash steps when dealing with assays of 2 μl or less. These assays will have to be in a simple mix-and-read format to enable us to screen 500,000–1,000,000 compounds within a period of one to two months.

RB: And what about the development and optimization times for these assays?

LB: What about them?

RB: Don't you think it will take longer to develop these assays?

LB: Why should it?

RB: Okay, let's take a step back for a minute. Let's say you have a situation, which appears to be somewhat common, where the initial assay is developed in one laboratory and the screening is performed in another

HTS laboratory. Are these assays, that are developed by a non-HTS laboratory, compatible with HTS?

LB: Are you kidding? I've had a couple of scientists come to me with a protocol where 2.5 ml of heptane are added in step 11!

RB: No!

LB: Although there are some labs that develop assays that are familiar with HTS assay technologies, in most instances, we have to do some assay optimization because they're not familiar with assay technologies, such as SPA or DELFIA (Dissociation Enhanced Lanthanide Fluorescent ImmunoAssay; Wallac Oy)[3], that reduce the number of assay steps and/or are homogeneous.

RB: So, you would agree that there is some 'gap' between the two labs?

LB: Yes, I agree.

RB: Okay. So, this gap will mean that some additional time will be required to adapt these assays to HTS, correct?

LB: I guess –

RB: And, don't you think this problem will be further exacerbated once, and I'm using your words here, miniaturized assays in 1,536-well plates become an integral part of the lead finding program? Won't additional efforts be required to adapt 96-well plate assays read in a plate reader to 1,536-well plate assays detected on a digital imager?

LB: Well –

RB: And this problem will become even more challenging when novel, developing technologies emerge. For example, let's say that you want to utilize a homogeneous fluorescent technology, such as HTRF (homogeneous time-resolved fluorescence; Packard Instrument Company)[4] or Lance (LANthanide ChElates; Wallac Oy). Who would be responsible for adapting this technology to the assay?

LB: Most likely this would occur in the HTS laboratory, since disseminating this information to every laboratory where initial assays are developed would be virtually impossible.

RB: All right. Based on this, don't you foresee a wider gap developing where the HTS laboratory will need to assume increasing responsibility for incorporating technology platforms that require considerable optimization?

LB: Why should it take any longer than existing technologies?

RB: Because, with some of the current homogeneous technologies, you're dealing with the transfer of fluorescent signal from a short-lived donor to a long-lived acceptor, and dealing with two labels (i.e. the donor and acceptor) will be more difficult than one label. With radioactivity, fluorescence, luminescence, time-resolved fluorescence and fluorescence polarization, you're dealing with detection of one signal. Optimization of energy transfer will be critical to the sensitivity of the assay. If assays continue to be developed in the therapeutic areas, there will be an increase in the technology gap that now exists.

LB: First, as experience with FRET (fluorescent resonance energy transfer) technologies has become more routine, dealing with two labels may at times pose a minor problem, but certainly not a major one. Second, proximity concerns with FRET do not appear to be an issue. Third, there are other technologies, such as SPA, in which both the bead and ligand are labeled, that do require two labels. And finally, in what sense do you see an increase in the technology gap?

RB: From the standpoint, as you describe, that homogeneous assays run in 1,536-well plates utilizing digital imaging will initially still be developed in 96-well plates. This will occur because it is unlikely that every laboratory that develops assays will be adequately equipped, in terms of instrumentation, for higher density plates. To compensate for this gap, there will need to be a shift in resourcing to optimize assays to incorporate these new technologies. The non-HTS laboratories cannot be expected to do this because the cost of replicating this effort in each of these non-HTS laboratories would be prohibitive.

CC: Okay. Let's get back to the issue of 9,600-well plates, which was touched on earlier. RB?

RB: All right. I'm concerned about screening in volumes of less than 2.0 μl. Do you really think that a significant number of assays can be screened in 200 nl volumes?

LB: Do I think all assays will be converted to these volumes? No, I don't, but I think there is the potential for a significant number of assays to be minimized to this extent. Look, three to four years from now, the sensitivity of assays may be increased by another tenfold. Did you think back in the early 1990s that we would be running assays in volumes <100 μl? Now, you're not even concerned about assays running in 20 μl volumes, only volumes that are tenfold less than this.

RB: It's not that I'm not concerned, it's just that I have a greater concern for 0.2 μl assay volumes. I'm concerned with any assay run with volumes less than 10 μl, due to evaporation issues, as well as issues concerning mixing, surface tension and other physical properties that may impact fluidics at this scale. The amount of evaporation at RT is significant in 1,536-well plates, where we know that >50% evaporation occurs in the outer wells within 30 min. And this will be even more critical in 9,600-well plates or denser formats. How do you expect to address these challenges?

LB: I acknowledge that evaporation is a significant problem. But we'll deal with it.

RB: How, by setting up an HTS laboratory in the tropical rain forest?

LB: No, we'll set up a large laboratory where an appropriate humidity level can be maintained.

RB: There you go, spending money again. You know, for the amount of money you want to spend, you'd better hope that a lot of lead compounds get to the market from HTS to justify these costs!

I have two issues with your suggestion. First, what do you think the effect of constantly high humidity levels will be on the instrumentation? I'm sure the effect of long-term condensation will not be insignificant. Secondly, don't you think the cost of an environmentally controlled room will be extremely high?

LB: First, we'll have to work with the manufacturers on the humidity problem. And second, the cost is inconsequential to the benefit of increased cost savings.

CC: Okay, let's move on to another topic. Earlier, RB brought up the issue of screening in singlicate determinations. LB, can you address this concern?

Cost issues

LB: All right. Let me provide another example, where our objective is to evaluate 200,000 compounds in 20 assays per year. Let's say, for the purpose of this discussion, that the total average cost of an assay in 96-well plates is $1.00 per well. The cost for running one assay in duplicate determinations (88 compounds per plate) would be $455,000 (Table 2). The cost savings realized for just one assay would be $227,000 for running singlicate instead of duplicate determinations. Look, I have to admit that I would like to screen using replicates. But the cost of running 20 assays a year in singlicate determination would amount to a savings of $4.6M. We need to remember that the goal is finding leads, rather than determining if a compound inhibited 20±2% vs 25±3% enzyme activity. Yes, I concede that perhaps a certain, small percentage of compounds may be missed by screening compounds in singlicate determinations. And I also concede that this additional data could provide a significant amount of information in the development of trend vectors or other computational models. But by not having to screen replicates, we can save 50% of the costs and complete screening in half the time.

RB: But this is not quality! I suppose next you'll be telling me you screen compounds in mixtures –

LB: Well, now that you mention it –

RB: What! You screen in mixtures too! Where did you say you got your degrees, off a matchbook cover?! How do you sleep at night?

LB: I don't; I have discussions with you. But seriously, it's a matter of cost. Look at the cost savings! Even if we screen using duplicate determinations to assuage your concerns, the cost of running 20 assays is now reduced to $1.8M instead of $9.1M.

I'd prefer to screen in single compounds per well. It just isn't practical at the present time. The value that can be potentially added by screening in singlicates is minimal compared to the cost, in terms of increased financial expense, in terms of the longer time required to identify a lead, or the lower number of compounds that would be screened. I submit that the added value, which would be realized in terms of quality, but not in terms of attaining the goal of rapidly finding leads, would be minimal.

RB: But what is the cost of the missed compounds?

LB: What missed compounds?

RB: Are any neurons firing in there? I'd bang my head against the wall, but that would wake 'him' up and end our discussion. The missed compounds are those false negatives that you'll never be aware of because of additive effects of the compounds in the mixture. Look, if you have one of five compounds in the mixture producing 80% inhibition of activity, a second compound producing a 40% increase in activity, and the other three compounds are inactive, the ability to identify that first compound as a hit would be missed because the overall activity would only be 40% inhibition.

Table 2. Effect of mixtures and replicates on screening costs

Number of compounds	Compounds per well: 1	5	Singlicate/ duplicate	Cost per assay	Total cost (20 assays)
200,000	X		D	$455,000	$9.1 M
200,000	X		S	$227,000	$4.6 M
200,000		X	D	$ 91,000	$1.8 M
200,000		X	S	$ 45,500	$0.9 M

Or, what would happen if you were looking for an agonist in a reporter gene assay, and you missed a compound that stimulated activity by 100% because another toxic compound inhibited 100% activity?

LB: Yes, I concede that there might be a problem. But, you have to consider other types of cost if we don't mix compounds, such as the cost of identifying a lead 3, 6 or 12 months too late, or the cost of missing a lead because you didn't have the capacity to screen all the compounds.

And furthermore, this doesn't even begin to show the time savings with miniaturization.

RB: Miniaturization again. All right; here, I have a problem. You know, it seems that there are some topics, such as miniaturization, where everyone becomes mesmerized by the concept, and does not consider any of the practical issues. You say you want to do all your screening in 384-well plates, is that correct?

LB: Yes, for now.

RB: And what are your expectations in terms of throughput?

LB: A fourfold increase in capacity, of course.

RB: You mean, you expect to be able to screen four times as many samples in the same period of time?

LB: Exactly.

RB: I guess you really haven't been in the lab for a while.

LB: What do you mean?

RB: Well, how long do you think it takes to determine radioactivity in each well of a 384-well plate compared to a 96-well plate using a scintillation counter with 12 detectors?

LB: Well, with the newer scintillation counters, it should take about the same time to count a 96- and 384-well plate.

RB: Well, maybe that instrument will exist in the future, but it doesn't exist today. No wonder you think there will be a 400% increase in productivity. It takes essentially the same amount of time to count one 384-well plate as it does four 96-well plates. Some time may be saved with the 384-well plates, since less physical manipulation of plates is required, but that time saving is relatively small.

LB: What about the other steps in the process of screening 384-well plates; for example, where instruments are available that can rapidly add reagents to these plates?

RB: In some cases, these steps are faster. However, one of the main rate-limiting steps is in compound distribution. You maintain your stock concentration of compounds in deep-well 96-well plates, correct?

LB: Yes, but I don't see the relevance...

RB: It's relevant in that it takes almost four times longer to distribute compounds from four deep-well 96-well plates into a 384-well microtiter plate, than it does to distribute compounds from one deep-well 96-well plate into a 96-well microtiter plate.

LB: So we'll just buy more pipetting devices.

RB: There you go again, spending more money.

LB: But the cost still justifies miniaturization!

RB: I don't deny that. All I am saying is that we should not miniaturize just for the sake of miniaturization. We need to consider what value we're adding by miniaturizing the assay, whether it be in terms of cost or increased productivity.

What about chip technologies?

CC: One last topic, gentlemen, before we conclude tonight's debate. What do you think the impact of chip-based technology will be on HTS?

LB: I believe that chip-based screening technologies will have a tremendous impact on HTS. It will provide us with the ability to evaluate millions of compounds in hundreds of assays for a fraction of the cost. I feel that it's a technology platform that we must start integrating.

CC: And you, RB?

RB: My question is, what will be the added value for integrating this platform? Up until the present time, most of the changes in technologies have amounted to add-ons to existing systems. Whether this meant

purchasing a new generation of liquid dispensers, or readers or imaging systems, they could be integrated into existing systems. Now, with chip-based technologies, your're talking about an entirely new platform. Is this platform really necessary? What value will it add to the existing program, and at what expense? In order for this technology to work, I think it's fair to assume that technology will already exist to screen one million samples, in two to three months, at a reduced cost due to miniaturization. Do we really need to increase the number of compounds, or further reduce the time of screening, or further reduce the cost, particularly if the price is going to be in the millions of dollars?

The other issue I have concerns the 'soluble' compounds we screen. Chip-based technologies may work well for compounds synthesized in chips, but how will this technology be used to screen our existing compound libraries? And furthermore, it's one thing to evaluate them in wells in microtiter plates. I think it will be another story to picture these compounds passing through microvessels. I keep seeing images of these partially soluble compounds lodged as boulders that block the microtubules. So at least from my perspective, I would not want to pursue chip-based assays.

CC: Colleagues, I want to thank you for an interesting discussion. I'm sure that once again, you've created a migraine. In order to help reduce the tension that has built up during this discussion, there is one thing I can suggest.

LB/RB: And that is?

CC: Random Screaming!

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