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AUTOMATED PATCH CLAMPING

setting a new standard for early hERG

By Dr John Comley

Ion channel targets remain a top priority for many Pharma and Biotechs with most looking to increase their screening efforts in 2006. The impact of automated patch clamping (APC) on ion channel screening is now evident, particularly in early non-compliant hERG liability testing, where APC is rapidly becoming the new 'gold standard' technology. User feedback on the overall performance and patch success rates of the APC systems they have implemented for hERG has generally been very positive. However, it is still possible to discern small differences in opinion between APC users with a strong electrophysiology background and those without, particularly with respect to the level of accuracy required for an APC system compared to conventional patch clamping; the minimum seal resistance needed and preferred approach to the reuse of whole cell preparations. Overall a greater consensus exists today on the use of APC than a few years ago. It is now apparent that the deployment of APC instruments into primary screening will be limited until next generation APC platforms emerge. Most restrictive today is the high cost of APC consumables (eg patch plates); the lack of suitable high-throughput instruments able to adequately address both voltage-gated and ligand-gated channels; and measurement dependence on cell quality. Despite these limitations, the market for APC instruments is predicted to continue to grow, with deployment of APC technology widely adopted throughout drug discovery in pharma, biotech and academic research labs. Reviewed in this article are the latest vendor updates on the status of their APC offerings. In addition, the prospects that next generation APC devices will emerge in the near future are discussed.

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Several years ago we reviewed high throughput electrophysiology[1] and detailed the technology developments that supported the then emerging field of automated patch clamping (APC). Since that article HTStec has monitored the growth of ion channel investigation through the publication of its annual Ion Channel Trends reports, the latest update of which was published in September 2005. The new report summarises current practices and technology preferences in ion channel screening and progress achieved towards implementing APC. One of the key differences with the latest report is the wider respondent sample across the full breadth of drug discovery, which gives the findings greater validity.

Across the board increases in ion channel screening metrics

The latest metrics on ion channel screening in Pharma and Biotech labs in 2005 and estimates for 2006 are summarised in **Table 1**, with increases predicted all round with respect to the number of targets investigated per lab; the number of primary screens conducted per lab; the number of wells screened per target; and the percentage of targets that are ligand-gated ion channels. The findings suggest that ion channel targets are still a top priority for many Pharma and Biotechs. The relative importance of ion channels, receptors and transporter targets under investigation today (2005) are presented in **Figure 1**. The results confirm the importance of hERG as the most studied ion channel with greater than three-quarters of all respondents investigating it.

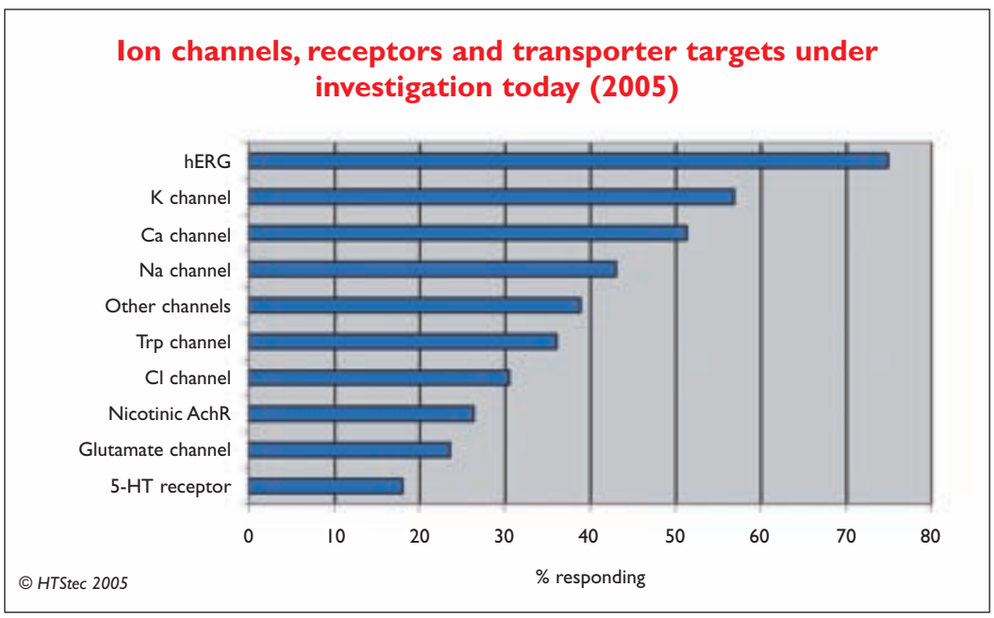
Automated patch clamping impacts on early hERG

The report also examines assay technologies preferences used today in the study of ion channels targets today (**Figure 2**). Fluorescence-based assays predominate in primary screening of full diversity libraries, with greatest use made of fluorescent-

Table 1: Pharma/biotech ion channel screening metrics

PARAMETER	2005	2006	% INCREASE
No. targets investigated/lab	7.9	8.1	2%
No. primary screens/lab	3.7	4.6	24%
No. wells screened/target (millions)	0.431	0.503	17%
Percentage of targets ligand-gated ion channels	28.7%	34.7%	21%

Figure 1



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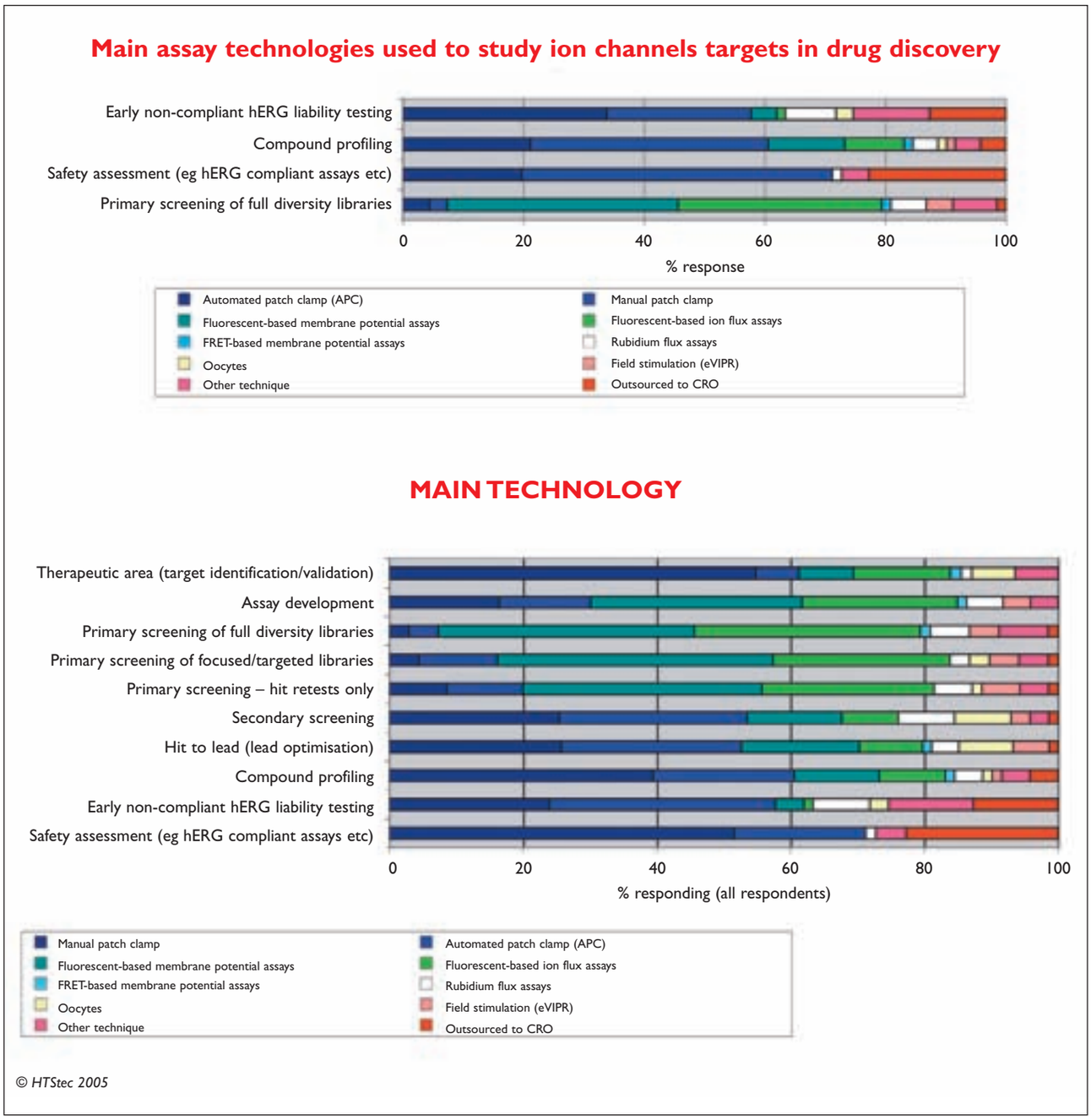

Figure 2

based membrane potential assays. These were followed closely by fluorescent-based ion flux assays and FRET-based membrane potential assays. However, a wider diversity of assay technologies are used for compound profiling, with greater than 50% of the respondents using some form of patch clamping (PC) (both manual and APC). For early non-compliant hERG liability testing, APC is the most popular technology, with respondents reporting they were generally happy with the overall performance and patch success rates of the APC systems they had implemented. In contrast, manual PC remains the preferred technology for ion channel safety assessment studies (eg hERG compliant assays). Interestingly, greatest use of outsourcing to a contract research organisation (CRO) was made by groups responsible for safety assessment and early non-compliant hERG liability testing. Many

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Figure 3

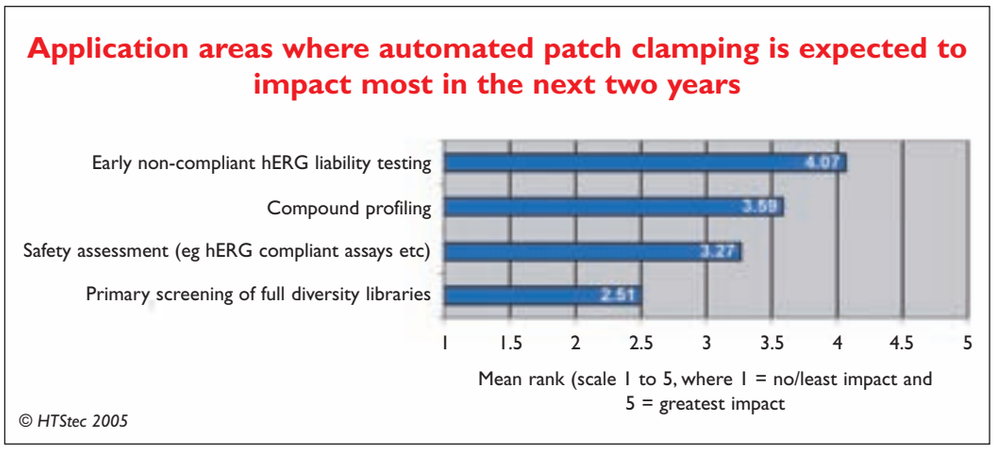
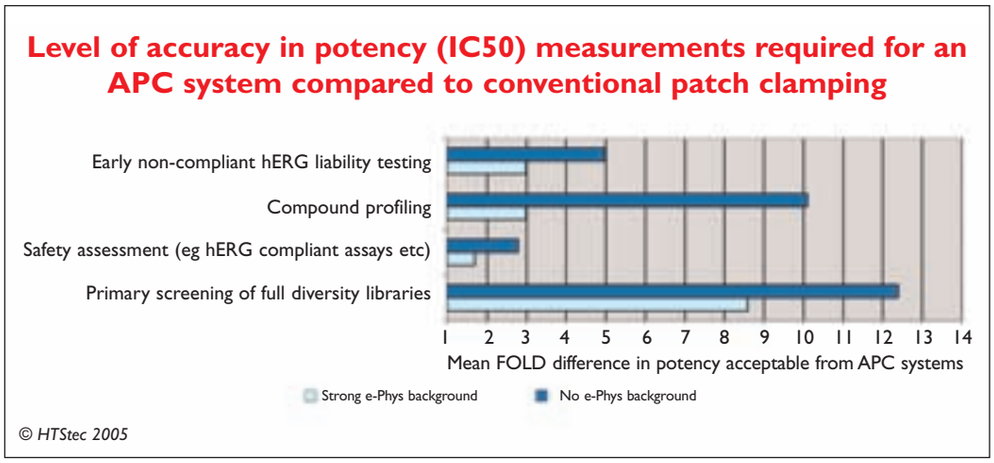


Figure 4



of these CROs (eg Aviva, Biofocus, bSys, ChanTest, Cytomyx and MDS Pharma Services) have implemented APC technologies over the past year and now offer hERG testing based on APC.

When respondents were asked to rank the application areas where APC is expected to make the most impact in the next two years, the findings mirrored the current use of ion channel assay technologies discussed above (**Figure 3**), ie greatest impact will be on early non-compliant hERG liability testing and compound (selectivity) profiling.

Mode of APC operation still a reason for diverse views

In 2003 we[1] reported that there were divergent opinions among traditional electro-physiologists and screeners as to the needed specification and the preferred mode of operation of APC devices. To see if these trends have continued, respondents to HTStec's survey were asked to categorise themselves on the basis of their electro-physiology (e-Phys) experience. It being assumed that those with no e-Phys experience probably had a screening background. In most respects there was no difference between the responses of these groups, although in those areas that impact on screening strategy some minor differences were still noted.

The first of these areas was in the level of accuracy in potency (IC50) measurements required for an APC system compared to conventional PC are presented in **Figure 4**. The results show that a greater similarity of results between technologies was demanded by safety assessment and early non-compliant hERG liability testing (maximum three-fold and five-fold difference respectively) versus primary screening (where up to 13-fold difference was accepted). However, those respondents with a strong background in e-Phys were more stringent in these requirements than those respondents with a non e-Phys background.

Another area of difference was in the minimum seal resistance required in a new APC instrument

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Figure 5

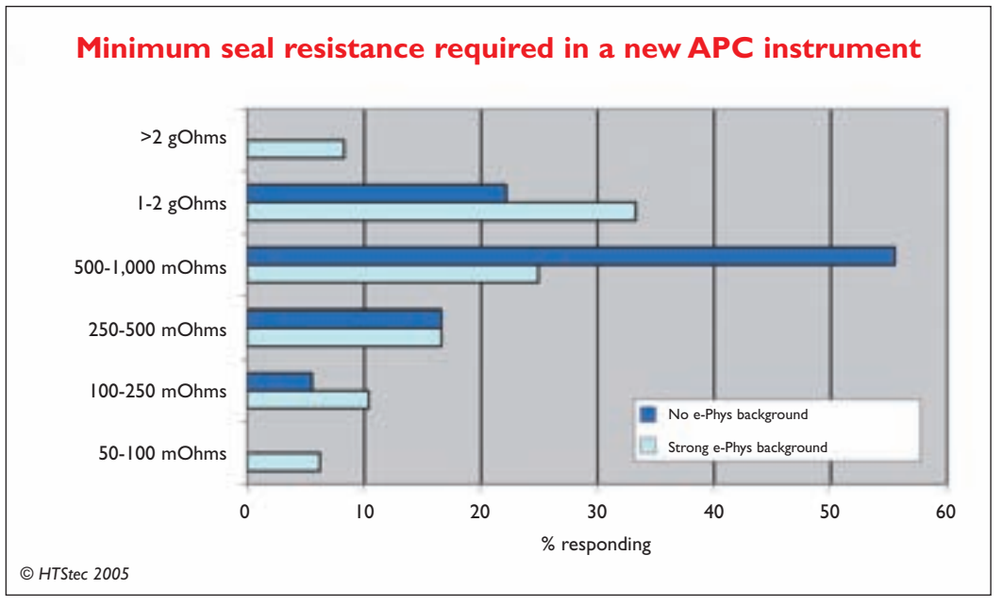
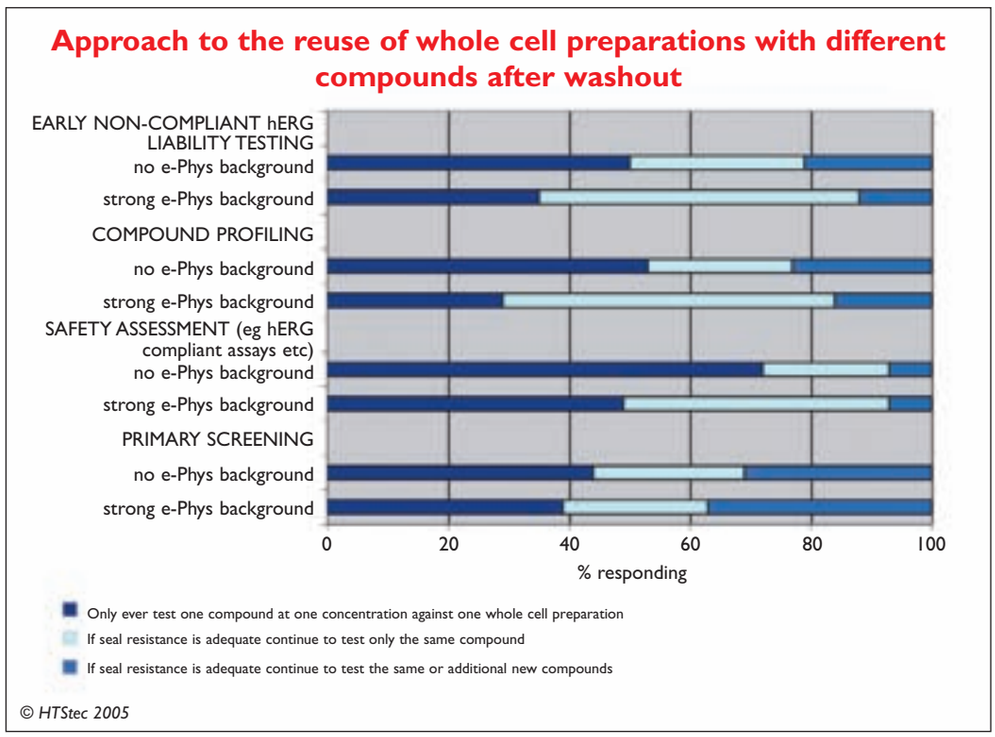


Figure 6



(**Figure 5**). The results show that the majority of respondents are seeking a whole cell resistance of between 500 mOhms and 2 giga Ohms (GOhms). However, once again those respondents with a strong background in e-Phys were more rigorous in their requirements as a greater proportion wanted a higher seal resistance (between 1 and 2 GOhms) than those respondents with no e-Phys background.

The final area that was the subject of much speculation in the previous review was how APC users would approach the reuse of whole cell preparations, with different compounds, after a washout (**Figure 6**). Users of conventional manual

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Table 2: Comparison of Automated Patch Clamp offerings

VENDOR	PRODUCT NAME	NO. OF MEASUREMENT SITES/CHIP	NO. OF SIMULTANEOUS MEASUREMENT SITES PROCESSED PER INSTRUMENT	AVERAGE CONSUMABLE COST (US$)[1]	COST PER DATA POINT ASSUMING SINGLE TEST (US$)[2]	COST PER DATA POINT ASSUMING MULTIPLE TESTS (US$)[3]	DATA POINTS/ 8H DAY ASSUMING SINGLE TESTS	DATA POINTS/ 8H DAY ASSUMING MULTIPLE TESTS
Cellectricon	Dynaflow Pro II DF8	1	1	95	na[4]	3	na[4]	128
Cellectricon	Dynaflow Pro II DF16	1	1	150	na[4]	2.3	na[4]	256
Cellectricon	Dynaflow Pro II DF48	1	1	260	na[4]	1.3	na[4]	768
Cytocentrics	Cytopatch 100	1	12	10	14	4	134	242
flyion	Flyscreen 8500	1[5]	3 or 6	5	7.5-10	2.5-3.3	100-500	100-500
Molecular Devices	Ionworks Quattro	384	48	240	0.75	na[6]	2,304	na[6]
Molecular Devices	PatchXpress 7000A	16	16	130	8.13	0.34[7]	240	240
Nanion	Port-a-Patch	1	1	5	5	1.25	40	100
Nanion	NPC-16s Double[8]	16	2	80	5	1.25	80	200
Nanion	NPC-16p	16	16	80	5	1.25	400	1,000
Sophion	Qpatch 16	16	16	120-160	~10	~4	500-1,000	200-500[9]
Sophion	QpatchHT	48	48	300-400	~5	~2	1,500-3,000	1,000-2,000[9]

NOTES
[1] estimated cost if product not yet available
[2] single test assumes only one compound at one concentration is tested against one whole cell APC preparation
[3] multiple test assumes that the same or additional new compounds are tested against one whole cell preparation
[4] although possible, single test is not typical for Dynaflow which is based on a multi-channel principle
[5] the Flyscreen robot, unlike the other APC systems, performs an asynchronous but parallel operation
[6] not applicable, as each compound addition has to be performed on a fresh cell
[7] assumes triplicate analysis at each concentration of an eight-point dose response curve
[8] configurations with four or eight parallel recording channels are also available
[9] assumes four-point dose-response curve per cell

PC methods have traditionally relied heavily on the reuse of whole cell preparations as a way of achieving greater throughput. Interestingly, the results showed that safety assessment measurements (previously reliant on the conventional PC) were viewed as the most exacting in their requirements with most respondents preferring to only ever test one compound at one concentration against one whole cell APC preparation. In contrast, primary screening measurements were viewed as the least exacting in their requirements with a greater proportion of respondents indicating if the seal resistance was adequate they would continue to test the same or additional new

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compounds. Those respondents with no e-Phys background showed a greater preference in all potential application areas of APC to only ever test one compound at one concentration against one whole cell preparation which is consistent with most primary screening approaches. In comparison those respondents with a strong background in e-Phys were more prepared to continue to test the same compound (eg at different concentrations) if the seal resistance was adequate.

Latest vendor update on their APC devices

Vendors and technology developers were asked to provide an update on their chip (patch plate) pricing and throughput potential for their latest APC product developments. The results presented in **Table 2** compare single test (assumes only one compound at one concentration is tested against one whole cell APC preparation) pricing and throughput with that from multiple tests (assumes that the same or additional new compounds are tested against one whole cell preparation). In contrast, survey respondents reportedly paid on average $8.76 per APC data point used in early non-compliant hERG liability testing (the average of all their use, ie both single and multiple tests), but thought that $2.66 would be a more reasonable cost. It remains to be seen whether APC chip prices will fall over the coming years to meet these market expectations.

The following additional information was chosen by APC technology vendors and developers to be highlighted.

Aviva Biosciences (www.avivabio.com) developed the SealChip, a planar electrode technology that is used in the PatchXpress automated high throughput electrophysiology instrument. Aviva's Sealchip combines unique surface chemistry with innovative design which enables automated voltage clamp conducted under GOhm seal conditions. Leveraging the expertise gathered through the Sealchip and PatchXpress development programmes, Aviva has started offering services for electrophysiology-based drug screening. Aviva

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Speed + Quality = Results

Cell based discovery technologies and services are what Aviva is about. We invented the SealChip technology and revolutionized ion channel research. Aviva can support your drug discovery from gene cloning and cell line generation through functional assays and screening. Our clients continue to rely on us to provide the highest quality, fastest and most professional service and we never disappoint them. When results matter, trust Aviva.

- Gene cloning
- Cell lines
- hERG screening
- Functional assays
- Ion channel screening
- GLP study capabilities

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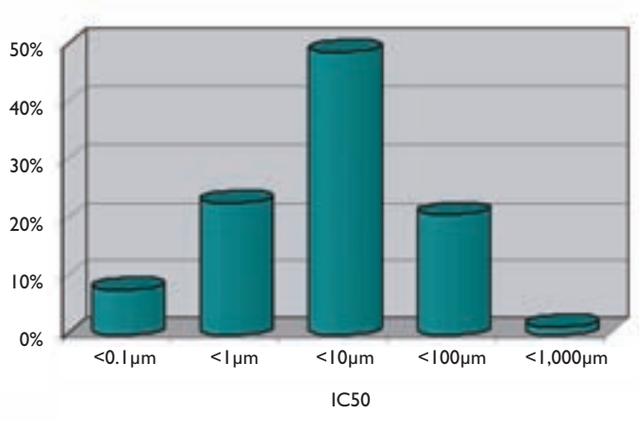
offers ion channel screening services to drug discovery and development companies by putting a bank of PatchXpress instruments and a variety of ion channel expressing cell lines to work for customers through its Pharma Services division. Aviva's Pharma Services division collaborates with drug development organisations of all sizes that chose to take advantage of the outsourcing option, either as a strategic decision not to internalise such specialised capabilities or because of capacity and time constraints. Access to automated electrophysiology based on true GOhm seals provide conclusive ion channel blocking results and has enabled many organisations to improve the quality and throughput of their *in vitro* safety testing resulting in compounds with better cardiac safety profiles. Past challenges associated with compound adsorption have now been addressed by innovative test protocol design. Aviva is also actively working on the next generation of SealChips.

Cytocentrics (www.cytocentrics.com) has now successfully tested the one-channel version of its CytoPatch™ instrument and demonstrated the generation of precise dose-response curves similar to manually obtained conventional PC data. The CytoPatch™ instrument has been validated by determining the effect of three known hERG channel blockers Terfenadine, Cisapride and Sotalol. The IC50 values were compared to data obtained with the traditional PC technique and literature values. The evaluation of Terfenadine is an excellent test of Cytopatch's compound handling and perfusion system as terfenadine is known as a 'sticky' compound which adsorbs to some materials. The literature range for manual PC acquired IC50 data is wide, ranging from 7.0nM[2] to 302.0nM[3]. The IC50 value for Terfenadine obtained with the CytoPatch™ technology was 29.6nM vs 8.3nM for manually PC data performed concurrently. The automatically determined IC50 values for Cisapride and Sotalol also showed an excellent agreement with literature and with manual PC data (ie Cisapride: 8.6nM vs 6.2nM, Sotalol: 361µM vs 281µM for CytoPatch™ vs manual PC data). In comparison with literature values the CytoPatch™ data are clustered in the lower range. This reflects the accuracy of the compound application and the microfluidic perfusion system. The sensitive detection of compound action is also insured by high seal resistances (Median: 2.3 GOhm), low leak current and high patch success rates typically around 75%[4]. Cytocentrics is currently proving ligand-gated ion channels on the CytoPatch, completing robustness testing and running customer samples in house before it plans the commercial launch of CytoPatch in 2007.

flyion's (www.flyion.com) patch clamp robot Flyscreen®8500 has now been available for more than two years and robustly enables between 100 and 500 independent whole-cell screens per day. The Flyscreen machine uses glass micropipettes similar to conventional patch clamp pipettes. However, the glass micropipettes are inverted placing the cell inside for sealing. Cell suspension containing only a few cells is automatically taken up from a cell hotel and dispensed into the back of the

With four PatchXpress instruments (top) available for client research, Aviva has the capacity to undertake a wide variety of discovery and screening programmes. Aviva Pharma Services group has evaluated thousands of compounds (bottom) since the introduction of the PatchXpress in 2003 and it is clear that hERG blockers are found throughout the discovery process

Distribution of potency for a random sample of 1,000 discovery compounds tested for inhibition of the hERG channel using automated patch clamp electrophysiology



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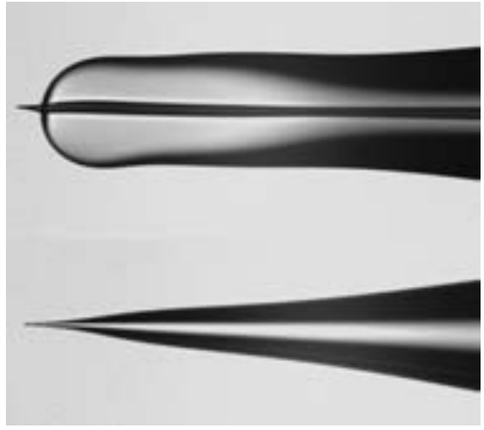
Cytocentrics CytoPatch™ instrument (left) with close-up (right) of chip inside the instrument. The chip has two concentric openings: the outer for cell positioning, the inner for patch clamping. The chip is made out of glass with embedded microfluidic channels

tip. The cells travel toward the tip of the pipette by gravity. Subsequent gentle suction draws a single cell into the end of the tip, forming a classical GigaSeal of 1–5 GOhm. Further suction pulses disrupt the membrane facing the tip. A plastic jacket moulded around the pipette allows for robotic handling. The machine contains up to six recording channels and each channel runs independently, so a new recording starts automatically as soon as a preparation fails. Temperature control of the recording socket allows for ion channel studies at physiological temperatures. Tip and liquid handling, seal formation, break-in and drug application are all performed automatically by the Flyscreen software; that analyses the data in real time and controls the flow of experiments. The system runs unattended for up to several hours. Glass tips are available with different shapes and dimensions. flyion's latest development is a new version of the FlipTip pipette, the so-called ChipTip. These tips have a wide shank and a flat seal area. ChipTips enable the Flyscreen®8500 to monitor ligand-gated ion channels. In flyion's standard tips, solution exchange takes about 60 seconds due to the unstirred layer. In the new ChipTip solutions are puffed directly on to the cell membrane yielding exchange rates of less than 50 milliseconds.



flyion Flyscreen®8500, with high power photograph beneath showing flyion's new ChipTip (upper) and a standard FlipTip (lower) for comparison

Molecular Devices Corporation's (www.moldev.com) two automated APC platforms, the PatchXpress 7000A and IonWorks Quattro, have paved the way in changing the face

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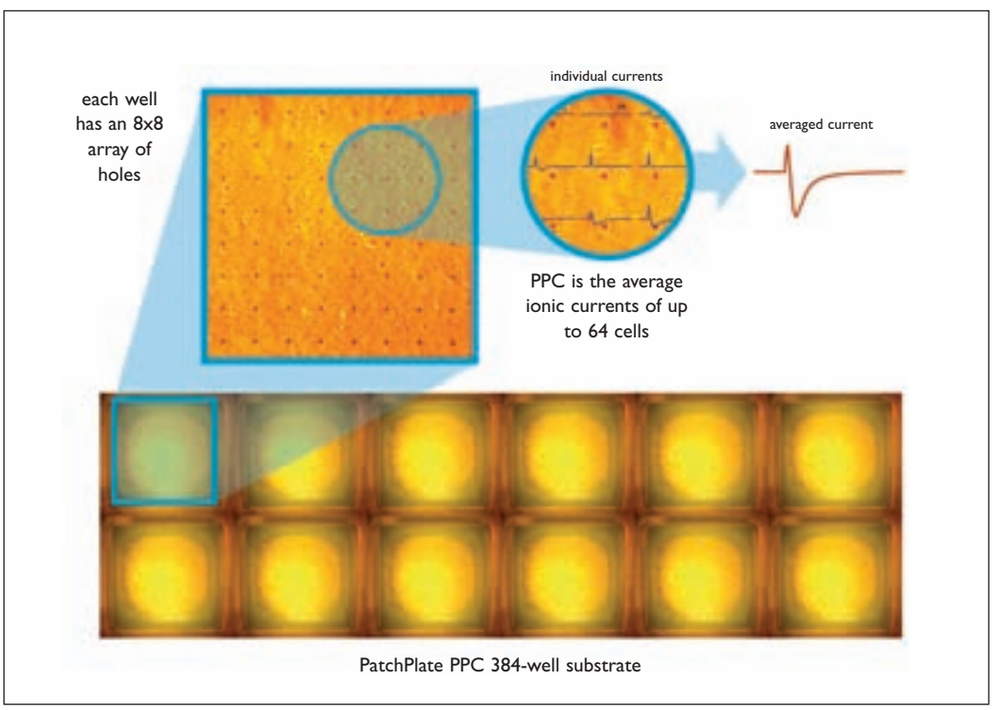
of ion channel drug discovery today. The IonWorks Quattro is a second generation automated patch-clamp system that uses a new technology developed by Molecular Devices called Population Patch Clamp™ (PPC). PPC uses 64 holes versus a single hole in each well of the 384-well PatchPlate. Therefore, the measured ion channel current from each PatchPlate well is an ensemble-average of a population of cells. With PPC technology, well-to-well variability is dramatically reduced and the success rate of obtaining a current measurement from each well is >95%. Using IonWorks Quattro system, scientists can measure up to 2,000 data points per day making the system ideal for secondary screening of hits from high throughput assays, primary screening of directed libraries, early safety assessment and pharmacology assays. The PatchXpress 7000A is an automated parallel patch clamp system that directly measures ion channel activity from up to 16 cells simultaneously. The unique SealChip™ planar electrode by Aviva Biosciences and the PatchXpress' rapid fluid delivery system supports high quality patch-clamp recordings of both voltage-gated and ligand-gated ion channels. True gigaohm seals and whole-cell recording enable the generation of research quality data from nearly any channel type. The system maintains most of the functionality of a conventional patch-clamp system, including capacitance and series resistance compensation and highly flexible voltage protocols. Recently, a new version of PatchXpress Commander (version 1.6) software was introduced. Some of the new features are: loops within procedures; multi-dose addition of test compounds; locking the compound robot for precise timing of ligand and drug delivery; membrane test between sweeps; resistive leak subtraction; and improvements to the cell health window. The new features in the software improve the system's ease-of-use and enhance its ability to assay ligand-gated ion channels. The PatchXpress 7000A system can be used for directed compound screens, lead optimisation/medicinal chemistry, and hERG safety testing for ADME toxicology.

Nanion (www.nanion.de) introduced its entry level device for automated patch clamping, the Port-a-Patch©, about two years ago. Nanion's Port-a-Patch© is not only the world's smallest patch-clamp setup with minimum foot print and low maintenance requirements, but also the cheapest way to access APC technology. The system uses Nanion's planar patch clamp chips and generates high quality data. The Port-a-Patch© enables fast fluid exchange on the chip, and is suitable for both voltage-gated and ligand-gated ion channels. It is a research grade instrument allowing for whole cell as well as single channel recordings with great experimental versatility. Even the exchange of intra-

Population Patch Clamp™ (PPC) technology used on Molecular Devices IonWorks Quattro



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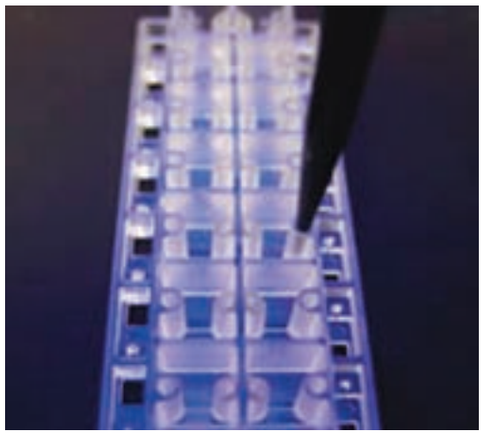

Nanion Port-a-Patch© with its chip mounting station (top left) and the software steered suction control unit. Electrolyte solutions and cell suspension are simply placed on the chip with standard pipettes and cell positioning and sealing is carried out automatically by the software. Nanion NPC©-16 fully automated workstation (bottom left) with two recording channels. The system can be upgraded to up to 16 channels. In all configurations, 48 recordings can be performed in unattended mode. Nanion NPC©-16 chip (bottom right) is a microstructured borosilicate glass substrate that is sandwiched within an 8x2 microfluidic cartridge. Different compounds or concentrations series for a dose-response curve are automatically applied to the patched cell by inserting the tip of the pipetting robot (shown) into the microfluidic channel of the chip

cellular solution is possible, enabling perfusion on both sides of the cell membrane. This possibility permits the investigation of ion channels regulated via internal binding sites for second messengers or other signalling pathways. It also enables perforated patch recordings by internal application of, for example, Amphotericin, Nystatin or Gramicidin. The Port-a-Patch© has been installed in many academic and industry labs with very positive customer feedback. It can be run by non-experts after a short training making patch clamp accessible to non-electrophysiologists. The same flexibility and high data quality is also achieved in a fully automated manner with the NPC©-16, Nanion's higher throughput patch clamp workstation due to be launched in April 2006. The system employs a robotic liquid handling environment for fully automated application of solutions, cells and compounds. The NPC©-16 executes a sequence of up to 48 patch clamp experiments in unattended mode. The NPC©-16 chips come in a neat microfluidic cartridge, which allow for fast and precise perfusion via microfluidic channels, eg a series of drug concentrations for dose response measurements. Nanion's consumable chips are vacuum packaged and stored dry at room temperature with a shelf life of at least one year. Success rates in forming gigaseals for the NPC©-16 as well as for the Port-a-Patch© depend somewhat on cell preparation used, but typically are around 60-80%. The Port-a-Patch© is proving a valuable tool for target validation, assay development alongside other higher throughput APC systems and in safety assessment. The NPC©-16 is expected to addresses these and other aspects of ion channel screening where moderate throughput is required.

Sophion Biosciences' (www.sophion.com) QPatch-16 automatically patch clamps up to 16 cells in parallel with the same high quality as conventional PC (ie gigaseals). Unlike any other planar PC system on the market, however, the QPatch provides at least four hours of unattended operation by virtue of its patented on-board cell preparation station. Unattended operation is a key feature for APC, which allows users to perform other important tasks during QPatch screens, such as analysing data, preparing solutions/compound plates or running experiments on a second QPatch system. The on-board cell preparation station (**Figure 1**) consists of stirring apparatus that keeps the cells in

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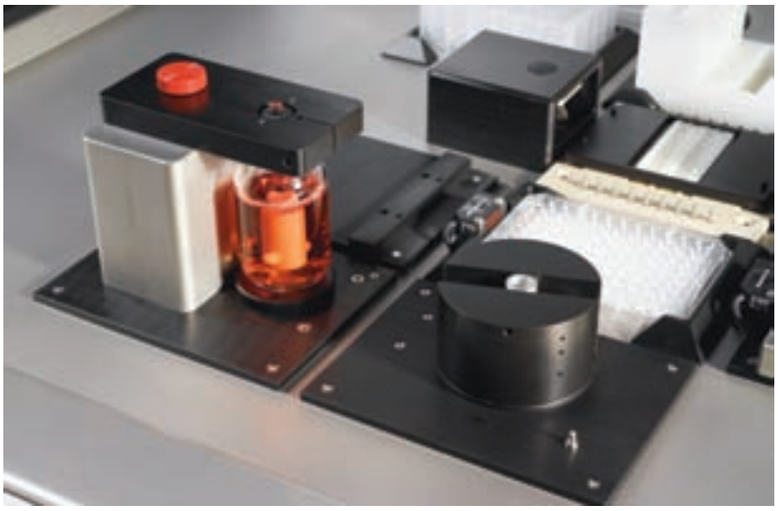
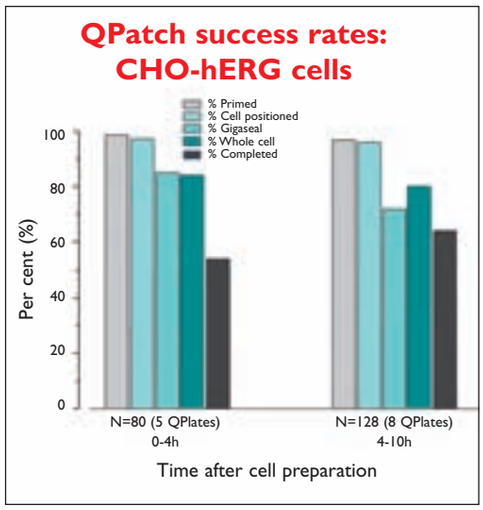
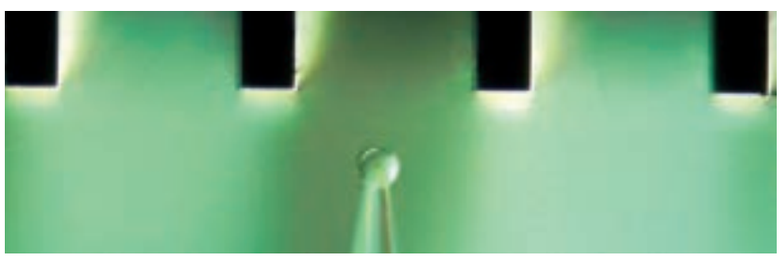




Sophion QPatch-16 workstation with on-board cell preparation device, showing the stirring apparatus (top left) and Q-fuge (on right, in front of the compound plate). Sophion QPatch success rates plotted as a function of the time after on-board cell preparation. Experiments were done with CHO cells expressing hERG potassium channels

suspension until the experiment begins. The QPatch pipetting robot aspirates ~1ml of cell suspension solution and transfers this to an Eppendorf tube in the 'Q-fuge' (on-board centrifuge). After spin-down, the cells are resuspended in extracellular solution and are ready to be applied to each of the 16 chambers of the QPlate. In some cases, the cells can be maintained on the QPatch for greater than four hours. **Figure 2** shows the results from a set of experiments with CHO cells expressing hERG potassium channels. After four hours, the success rates for gigaseals and whole cell recordings were both ~80%. The success rate for completed experiments (ie dose-response experiments) was ~50%. This success rate is similar to typical 30 minute experiments with the QPatch[5] and published values for automated patch clamp[6]. Interestingly, after 10 hours in suspension on the QPatch the success rate for completed experiments actually climbed a bit to just over 60%. The QPatch 16 was developed with more channels in mind and a 48-channel version (the QPatch HT) incorporating feedback from collaborators is expected to be available in Q4 2006. The QPatch HT is poised to be the world's first 48-channel gigaseal patch clamp system with on-board cell



Cellectricon Dynaflow Pro II System (top) is easily installed on an existing patch-clamp rig and consists of: Scan Stage, Pump, Commander Software, Controller and Microfluidic Chips (8-, 16- and 48-channels). A close-up (bottom) of a patch-clamped cell and the laminar flow zones at the channel outlets of the Dynaflow Microfludic Chips

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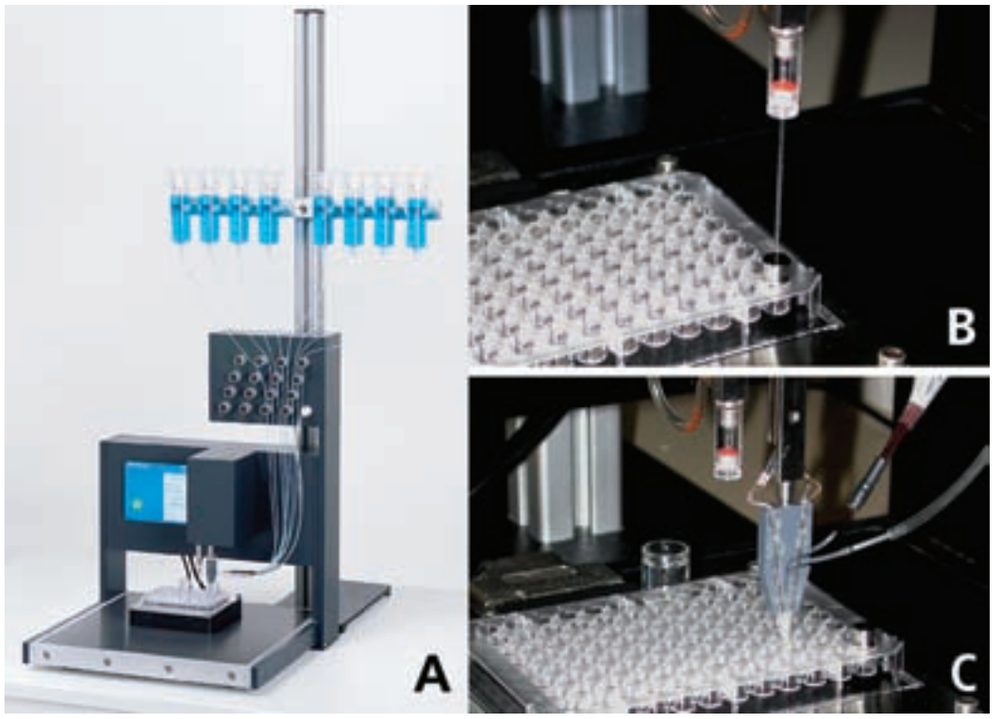

Multi Channel Systems' Roboocyte system for automated ion channel screening. The well plate with 96 oocytes is mounted on to the robot (A, shown without optional liquid handling station). The Roboocyte fully automatically injects the target ion channel (B) and performs the recording protocol (C) according to the specifications of the user

preparation and an eight-channel fluid handling robot, instead of four, to keep up with the higher throughput enabled.

Alternatives to automated patch clamping

The advantages of APC systems need to be put in context, for although they do indeed offer increased rates of compound testing, this improvement comes at a price, namely reduced recording quality and a loss of flexibility and control during the experiment. For example, it is still not possible to use native cells on APC systems, and not all APC systems offer fast and continuous laminar buffer flow that is required for the recording from ligand-gated ion channels. Consequently, the need for high-throughput electrophysiology systems that support the advantages of manual setups and enable screening of all types of ion channels at low cost while still maintaining the high information content and data quality offered by classic PC screening still exists.

Cellectricon's (www.cellectricon.com) Dynaflow™ Pro II is a chip-based system that is easily installed on to an existing PC rig and turns it into an easy-to-use high throughput electrophysiology system. Dynaflow enables sequential rather than parallel testing of compounds on ion channels by high-speed translational scanning of a single PC cell across a laminar stream of solution environments created by the Dynaflow microfluidic chips. The translational scanning of the microfluidic chip, causing a PC cell to sample the discrete zones of drug solutions, is controlled by a computer controlled motorised scan stage. Because Dynaflow enables complete control over the solution exchange around a PC cell, and markedly prolonged PC recording times and increased seal resistance, the system offers unprecedented data quality and experimental control while increasing the throughput. The core technological component in the Dynaflow system is the microfluidic chips. For maximised flexibility in assay design, the Dynaflow chips are available in eight-channel, 16-channel and 48-channel configurations. The chips are designed to be used with any patch configuration, any cell type, any receptor and any ion channel. All of the chips have an effective run time of more than 100 minutes, thus facilitating analysis of multiple cells on each chip. The 48-channel chip is designed for achieving maximum throughput in screening applications or for extracting multiple compound dose-response measurements on one chip. Because of the large number of channels, the chip facilitates highly complex assays. Dynaflow 16-channel chip is designed to extract a full dose-

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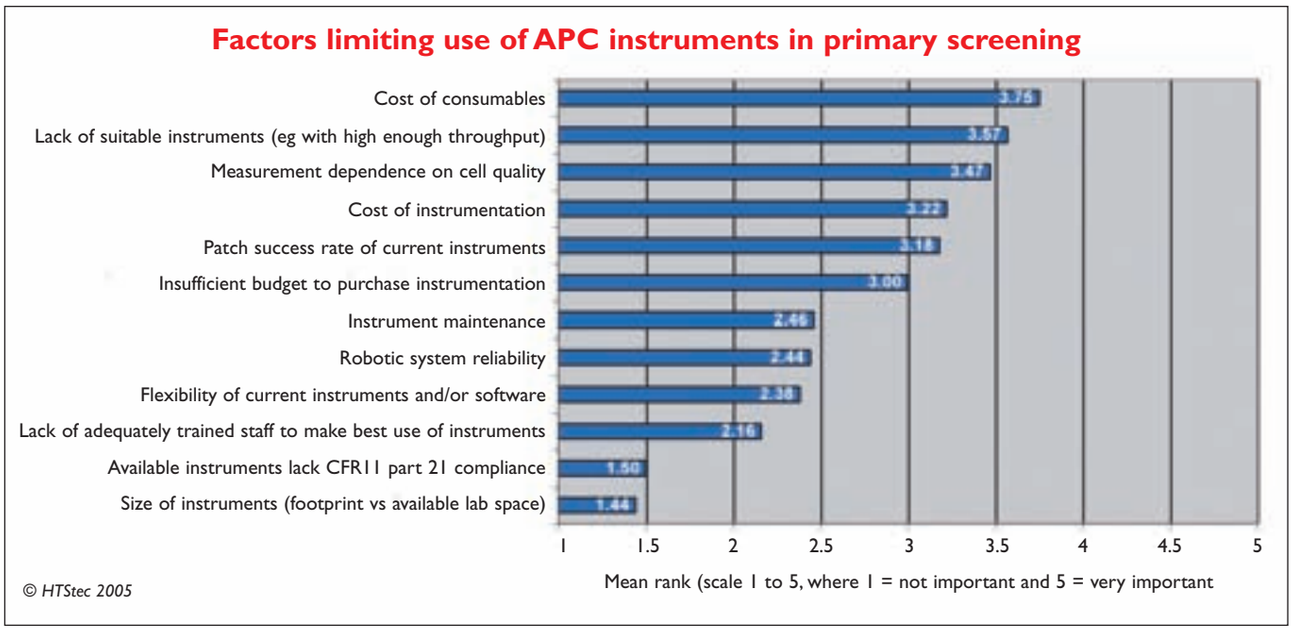

Figure 7

response measurement with washout between substances from a single cell or screening of up to 15 substances per chip. The recently launched Dynaflow eight-channel chip is designed for cumulative dose-response work and/or screening of slow acting compounds on non-desensitising ion channels and is well matched for safety pharmacology studies. Consequently, the Dynaflow System is ideally suited for ion channel assays across drug discovery process where optimal data quality really is the key.

The Xenopus oocyte expression system and the Two-Electrode-Voltage-Clamp (TEVC) recording method provide flexible alternatives for studying all classes of ion channels. This assay requires that the mRNA/cDNA encoding the target receptor is injected into Xenopus oocytes a few days before the planned experiment and is particularly useful when assessing large numbers of receptors, eg during compound selectivity profiling. **Multi Channel Systems**' (www.multichannelsystems.com) Roboocyte is the only fully automated oocyte screening system in the market that allows continuous operation in a 96-well plate format without user intervention. Recently Multi Channel Systems has improved the graphical user interface of the Roboocyte system and extended the options for recording protocols such that it is now possible to predefine user prompts for experimental settings that vary from run to run, which can be modified by the user on screen before starting the run. User defined variables facilitate the design of recording protocols tailored to the requirements of individual experiments. Additional analysis features have been implemented, like multiple regions of interest, the automated generation of dose response and I/V curves, and extraction of EC/IC50 values. Overall, the Roboocyte system now comes very close to the walk up/walk away automated solutions sought after by pharmaceutical companies. A major technical challenge recently met was the redesign of the integrated digital TEVC amplifier. The main scientific goal for developing the next amplifier generation was to increase the rise time and the sampling rate for supporting stable recordings from the class of fast sodium ion channels, as well as further advancing the automation, user-friendliness and clamp performance in general. Validation studies on the SCN5A channel (unpublished data) demonstrated the improvements: The output/injection current for fast voltage jumps is now ±65 µA (was: ±32 µA). The rise time (10-90%) of the amplifier is now safely below 1ms, typically 500µs (was 2ms). The sampling rate is now 10kHz (was 2kHz). In summary, the new amplifier design has successfully extended the use of the Roboocyte to the class of fast sodium channels.

Prospects for primary screening

Many survey respondents noted that they would like to use APC readouts for primary screening, but they believe that the deployment of APC instruments into primary screening will continue to be

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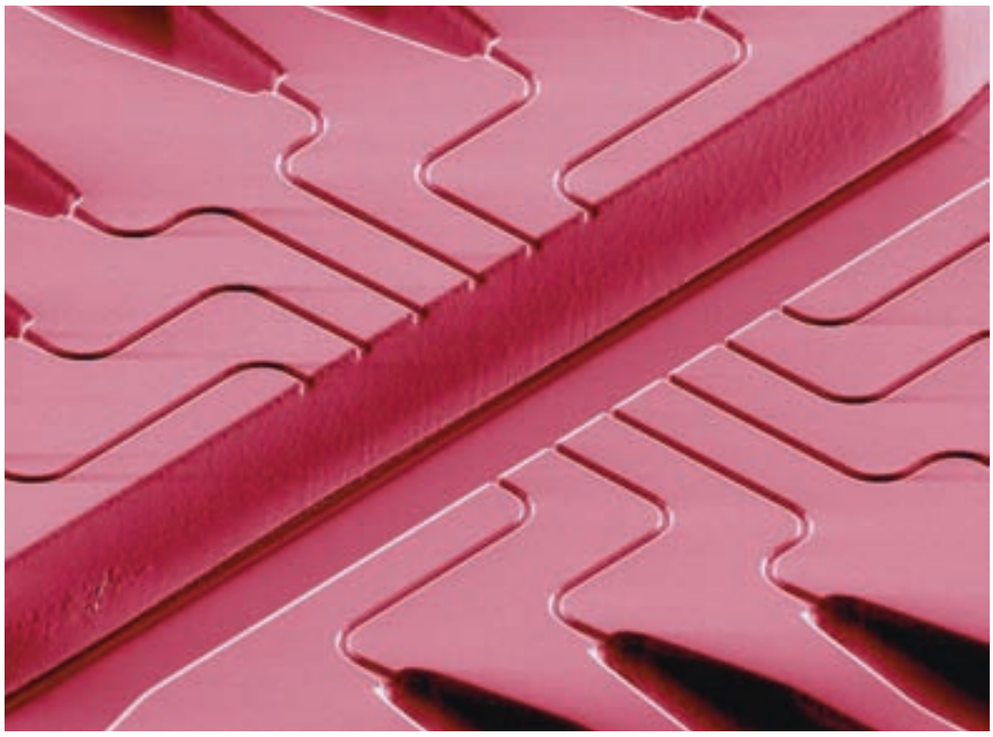
limited for the foreseeable future. The relative ranking of factors limiting use of APC instruments in primary screening is presented in **Figure 6**. Most limiting is the high cost of APC consumables (ie patch plates); the lack of suitable high-throughput instruments able to adequately address both voltage-gated and ligand-gated channels; and measurement dependence on cell quality. Evidence that unmet needs still exists among APC offerings is reflected in the fact that survey respondents preferred ideal plate format for an APC patch plate is not single patches, 16 or 48-well patch plates where most of the existing offerings are focused, but 96 or 384-well patch plates (**Figure 7**). One advance that has potential to impact on primary screening is being developed by **Panasonic Factory Solutions Company**, (http://panasonic.co.jp/pfsc/en/) in collaboration with its strategic life science partners. Panasonic is leveraging its semiconductor and factory automations technologies to develop a novel ultra high throughput planar patch clamp screening system that will address many of the unmet needs of primary ion channel screeners identified in HTStec's survey. The new 384-plate-based system, which will be sold under the trade name of GigaPatch™, is expected to be available to customers by the end of 2006.

Although recent APC developments based on planar geometry have increased and offer potential to further improve the throughput of electrophysiology; most still involve expensive fabrication steps and offer none or limited ability to visually access the cells being studied. In order to improve visualisation and the control of cell position with disposable microfluidic devices, the **BioPOETS Group** at UC Berkeley[7] have developed a simple alternative patch clamp technique based on soft state microfluidic junctions between a main chamber and lateral recording capillaries, which is fabricated by micromolding of polydimethylsiloxane (PDMS) (see photo). The soft and transparent elastomeric properties of PDMS substrates eliminate the need for vibration isolation and allow direct cell visualisation and manipulation using standard microscopy. Microfluidic integration allows capillaries to be arrayed at high density format to multiple hydrodynamic single cell trapping sites. The geometry of the recording capillaries permits high quality stable whole-cell seals despite the hydrophobicity of the PDMS surface. Using such a device, BioPOETs has been able to demonstrate reliable whole cell recording of mammalian cells on an inexpensive disposable microfluidic platform. Recordings of activation of the voltage sensitive potassium channel Kv2.1 in mammalian cells compare well with traditional pipette recordings.



SEM of 12 channel patch clamp array chip developed by the BioPOETS group at UC Berkeley

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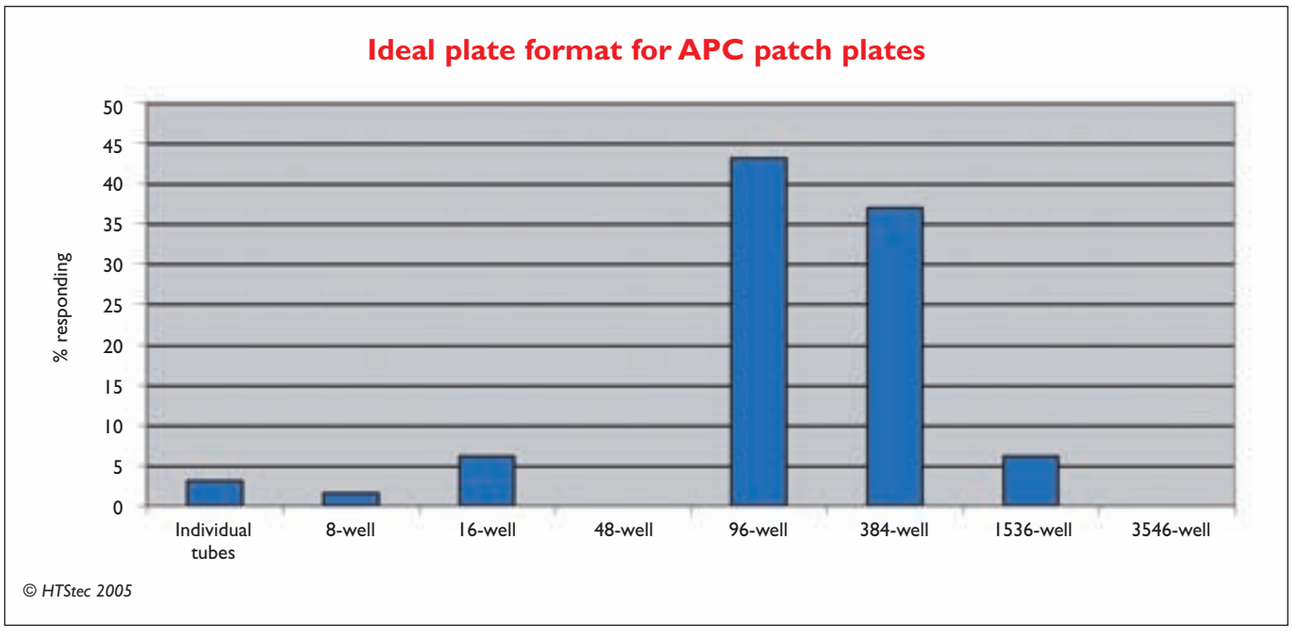

Figure 8

The results make possible the future integration of whole cell electrophysiology with easily manufactured microfluidic lab-on-a-chip devices containing a very high density of patch sites.

Without taking into account the impact that next-generation instruments, such as the GigaPatch™, might have, the market for APC instruments is predicted to continue to grow significantly, with projected sales of more than 200 APC units globally in 2006. The deployment of APC technology is increasingly widespread throughout pharma, biotech, CRO and academic research labs and its applications are set to broaden well beyond safety screening and into ion channel-targeted drug discovery, opening fertile ground for therapeutic opportunities. DDW

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