Optimizing the hERG Assay on the PatchXpress 7000A System for Compounds that Demonstrate Non-specific Binding

Iris Yang#, Naibo Yang#, Cathy Smith-Maxwell#, Claire Quinn*, Kirsty Macfarlane* and David Yamane#

#Molecular Devices Corp., 3280 Whipple Road, Union City, CA 94587

***Quintiles Limited, Edinburgh, Scotland, EH14 4AP. UK**

Abstract

Non-specific binding of compound can affect the potency measurement from both conventional and automated patch-clamp assays. Because of this, pre-cautions need to be taken to ensure this is minimized or eliminated. Eight compounds, astemizole, pimozide, dofetilide, cisapride, terfenadine, flunarizine, quinidine and imipramine were used to evaluate the effect of different assay conditions to overcome the effects of non-specific binding in our hERG assay on the PatchXpress 7000A system. To achieve the best IC50 values, in addition to optimizing instrument parameters to minimize non-specific binding, care had to be taken in all phases of compound preparation. Compound binding to plastic container need to be minimized. Buffers used for the assay also need to be optimized. In this poster, we present the assay conditions found that generated the best IC_{50} values on the PatchXpress 7000A as well as the assay results for these compounds.

Figure 1. **PatchXpress Automated Parallel Patch Clamp System.** Photograph of the PatchXpress 7000A instrument.

PatchXpress features:

- Gigaseals (cell-attached resistance > 1 Gohm) to start each recording
- True whole-cell recordings
- 50-90% seal/whole-cell success rates, depending on cell type
- Low access resistance (Ra):
  Reduce with feedback using added suction
  Electronic Ra correction
- Whole-cell capacitance compensation
- Easily test 240 compounds per 8-hour day
  (or 60 compounds at 4 concentrations)
- 16 independent channels record simultaneously
- 8 dual-channel MultiClamp 700A patch clamp amplifiers
  (proven research-quality amplifier)
- High-fidelity 16-channel input/output digitizer
  (proven technology used in MDC/Axon Digidata 1322A)
- Rapid and intelligent fluidics
- Highly flexible and straightforward GUI for setting up protocols to test a wide range of **voltage-gated** and **ligand gated channels**

16-Channel Wash Station

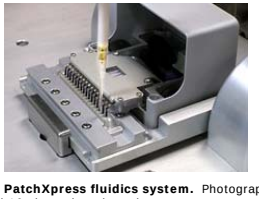

Figure 2. **PatchXpress fluidics system.** Photograph of the pipetting robot and 16-channel wash station.

Separate wash station perfuses each cell independently with buffer to wash out compound and/or ligand between additions and frees the pipetting robot to be used only for drug or ligand addition and not for washes.

The independent wash station makes it possible to remove excess cells and to wash cells for as long as it takes to remove drug or ligand.

IC_{50} values correlation between references and PatchXpress 7000A system

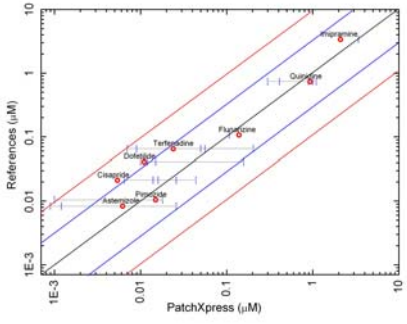

Figure 3. **Range of IC_{50} values found in the literature and IC_{50} values determined using the PatchXpress 7000A system.** The black horizontal lines indicate the range of IC_{50} values found in the literature for reference compounds. The blue hash marks show IC_{50} values taken from the literature. The red circles are mean IC_{50} values determined on the PatchXpress 7000A system. All values are generated from HEK-hERG cell line with high-chloride recording buffer (as indicated in figure 5). The parallel blue and red lines show 3 and 10 fold difference off the line of unity. The X-Y correlation was plotted against the average values of published IC_{50} values.

	IC_{50} (μM)	
	PX7000A	**Conventional**
Astemizole	0.0062	0.0009-0.026
Cisapride	0.0054	0.014-0.044
Pimozide	0.015	0.001-0.018
Flunarizine	0.139	0.107
Quinidine	0.931	0.300-1.1
Terfenadine	0.024	0.007-0.204
Dofetilide	0.011	0.010-0.158
Imipramine	2.08	3.4

Table 1. Range of IC_{50} values found in the conventional patch clamp method and IC_{50} values determined using the PatchXpress 7000A system

How to get the best IC_{50} values

(with both conventional and automated patch clamp system)

- Make drug solutions fresh just before each experiment
- Use DMSO for initial dilutions of all solutions and final concentrations of 0.1% DMSO
- Sonicate less soluble compounds to get them into solution
- Use glass or glass-coated vials and multi-well plates for all drug solutions
- Use buffer solutions for the experiment that yield the best IC_{50} values, for eg. High-chloride internal solutions
- Add fewer cells and/or washout remaining cells before adding drug
- Apply drug rapidly to promote rapid mixing near the cell
- Use multiple additions of the same compound and concentration
- Allow currents to reach steady state before making measurements
- Optimize voltage protocols for the state of the channel bound by the drug
- Correct current recordings to remove the leak current before doing analysis

Effect of plate material on IC_{50} determinations

Quintiles Terfenadine optimization

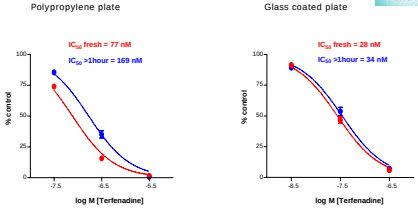

Figure 4. **Comparison of polypropylene and glass-coated compound plates** The IC_{50} value for terfenadine was 2.7-fold higher when polypropylene compound plates were used versus glass compound plates. Incubation of terfenadine in polypropylene compound plates for an hour resulted in an additional 2.2-fold increase in the IC50 value measured. Incubation of terfenadine in glass compound plates resulted in only a small increase in the IC_{50} value measured. (Figure provided by Quintiles, CHO-hERG cell line)

Buffer solutions alter block by terfenadine

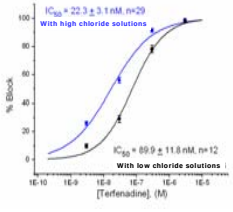

Figure 5. **Effect of saline solution composition on IC50 values.** High-chloride internal solutions provide the best IC_{50} values.

Composition of solutions

High-chloride (blue dose response curve)

Internal Solution (in mM): 130 KCl, 1 $MgCl_2$, 5 EGTA, 10 HEPES, 5 Mg-ATP (pH adjusted to 7.2 with KOH; osmolarity ~280 mOsm)

External Solution (in mM): 137 NaCl, 4 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 D-glucose (pH adjusted to 7.4 with NaOH; osmolarity ~291 mOsm)

Low-chloride (black dose response curve)

Internal Solution (in mM): 20 KCl, 8 NaCl, 110 K Glutamate, 1 $MgCl_2$, 10 EGTA, 10 HEPES, 4 Mg-ATP (pH adjusted to 7.2 with KOH; osmolarity ~280 mOsm)

External Solution (in mM): 137 NaCl, 8.06 Na_2HPO_4, 0.33 Na Pyruvate, 2.68 KCl, 1.47KH_2PO_4, 0.68 $CaCl_2$, 0.49 $MgCl_2$, 5.55 D-glucose (pH adjusted to 7.4; osmolarity 290 mOsm)

Mimic continuous flow conditions on the PatchXpress 7000A system

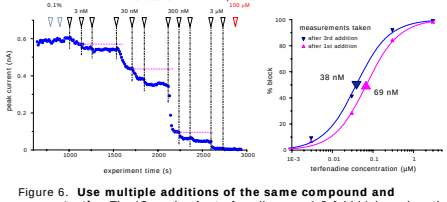

Figure 6. **Use multiple additions of the same compound and concentration** The IC_{50} value for terfenadine was 1.8-fold higher when the measurements were taken from the first addition versus the third addition. Adding compound multiple times at each concentration to mimic continuous flow conditions enables the PatchXpress 7000A system to achieve the best IC_{50} values.

Block of hERG by flunarizine requires sufficient time to reach steady state

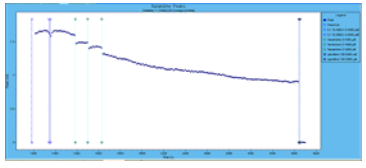

Fig 7. **Block of hERG by Flunarizine** Flunarizine act slowly on hERG channels. The low concentrations (≤ 100 nM) may take ~30 minutes to reach steady state block.

Monitor channel activity by PatchXpress software

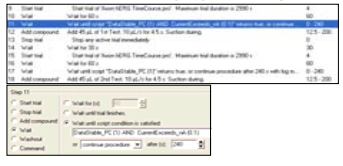

Fig 8. **The function of DataStable_PC on the PatchXpress software** The PatchXpress software can monitor the change in current over time and automatically decide, based on user-defined criteria, when steady state has been achieved.

Summary:

- PatchXpress provides high quality data and the IC_{50} values from PatchXpress are comparable to findings from conventional patch clamp method.
- Drugs bind to polypropylene plates over a 1 hour incubation period leading to modest decreases in potency.
- Drugs also bind to glass plates over a 1 hour incubation period implying that for the best IC_{50} values, drug plates should not sit in the instrument for several hours at a time even in glass plates.
- For the hERG channel, our data suggest that high-chloride internal solutions provide the best IC_{50} values.
- Use multiple additions of the same compound and concentration on the PatchXpress 7000A system to mimic continuous flow conditions, as in conventional patch clamp systems, to achieve the best IC_{50} values.
- Consider longer wait times for lower concentrations to ensure the response reaches steady state block.

Conclusion

Using the PatchXpress 7000A system we were able to identify several factors that affect the IC_{50} values, especially for hydrophobic compounds. By incorporating the recommended changes into our hERG assay, IC_{50} values for the reference compounds were within the range of IC_{50} values reported in the literature and were typically within three-fold of the lowest values in the literature.

Acknowledgements:

We would like to thank Cytomyx for providing the HEK-hERG cell line.

Molecular Devices