

Screening Compounds that Modulate Ionic Currents Using the IonWorks™ HT System

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Abstract

The IonWorks HT instrument is an automated high-throughput voltage clamp system that measures whole-cell currents from multiple cells in parallel using 384-well PatchPlates™. This system has the robustness required for single point screening of compounds that modulate ionic channel activity as well as the fidelity which allows IC₅₀ determinations. We performed a screen for hERG activity using six 96 well compound plates containing 80 “unknowns” each. Within these “unknowns” three wells were randomly spiked with a hERG inhibitor. Z-scores were calculated for each experimental run and are presented. Inhibition curves were generated for compounds with hERG activity (dofetilide, E4031 and terfenadine). Data is also presented for a fast Na⁺ channel (Na_v1.5) including z-score determination and pharmacology. This system provides an enormous opportunity to screen pharmaceutical compound libraries directly at the electrophysiological level and more effectively exploit ion channel targets.

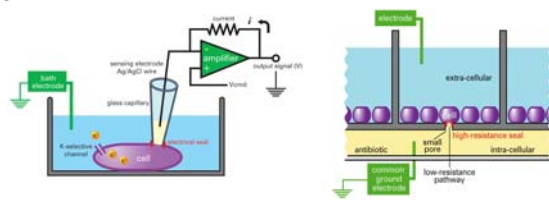


Fig 1. **Conventional vs. planar patch clamp recording.** Conventional patch clamp recording (left) and the planar patch clamp configuration used on the IonWorks HT instrument (right). The configuration used on the instrument utilizes a common electrode in the lower (common) chamber. Voltage is controlled and ionic currents are measured by one of the forty eight pins in the electronic head (see below) inserted into the upper compartment. The entire PatchPlate is read in 8 groups by the 48 electrodes in the electronic head. Electrical access to the interior of the cell is achieved by circulating perforating agent (Amphotericin B) in the lower chamber.

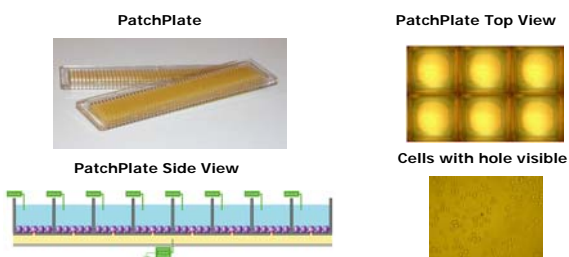


Fig 2. **Configuration of the PatchPlate.** The PatchPlate (upper-left) is a 384 well plate (8 x 48) with a ~12 µl working volume. Cartoon of a cross-section of the patch plate (lower-left). Photograph of six wells of the PatchPlate (upper-right) and substrate including the cells surrounding the hole (lower-right)

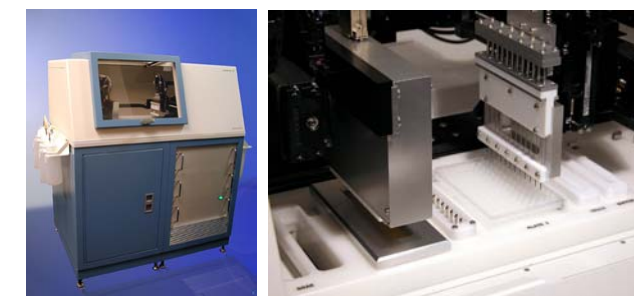


Fig 3. **The IonWorks HT instrument.** A photograph of the instrument (left) and the working surface of the interior of the instrument (right). The electronics head, fluidics head, positions for the PatchPlate and compounds plates as well as the electronic and fluidics head wash stations are shown. The instrument is available for viewing in the Molecular Devices booth at this meeting (booth #722).

Defining the “success rate” of experimental runs

Success Factor	Kv 1.5 - CHO			Nav h1 - CHL			hERG - CHO		
	Dropouts	Remaining	% Success	Dropouts	Remaining	% Success	Dropouts	Remaining	% Success
Hole Test	1	383	99	1	383	99	2	382	99
Seal Test	31	352	92	21	362	94	46	336	88
Current Amplitude	22	330	86	16	346	90	39	297	77
Stability	10	320	83	30	316	82	17	280	73

Table 1: The success rate is defined as the number of wells with usable recordings obtained during a run/384 wells. Cells are “dropouts” or considered failures if the well fails to prime (hole test), the cell fails to seal, channel expression is too low, or the Pre-/Post-read exhibits less than 20% stability during a mock compound run. The overall success rate for each cell line is shown in red.

(CHO = Chinese Hamster Ovary Cells, CHL = Chinese Hamster Lung Cells)

Command voltage editor & data acquisition

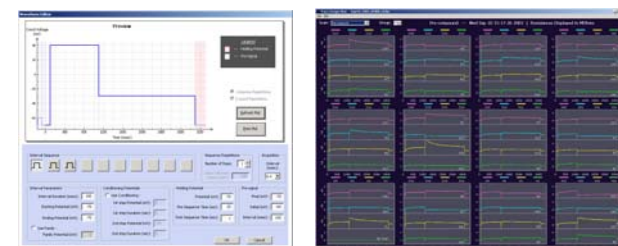


Fig 4. **Data acquisition** The command voltage editor is shown (left) and a screen shot of the raw data during data acquisition (right). The PatchPlate is read in 8 groups of 48, one of these 8 groups is displayed.

Post acquisition data filtering and hit configuration

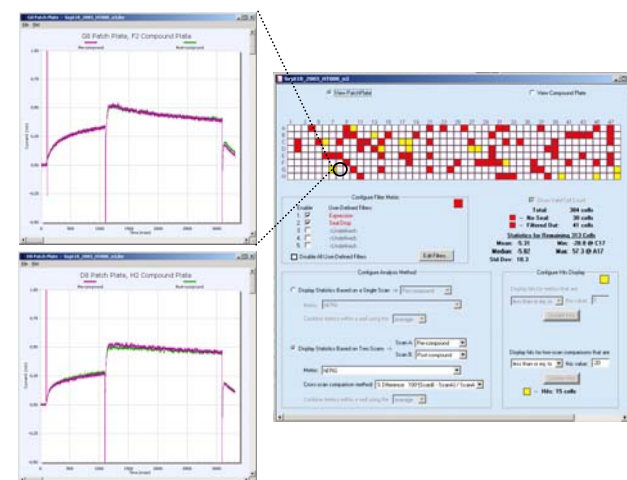


Fig 5. **Post acquisition data display.** The PatchPlate as it is displayed at the end of an experimental run (right). Left: Superimposed pre- (purple) and post-compound (green) current recordings of hERG channels from two different wells. The superimposed currents from any well in the PatchPlate are automatically displayed by double clicking on the individual well. Filters can be set to exclude currents based on their current magnitude, seal resistance, or the size of any baseline offset. Wells that contain cells that are filtered out are colored in “red”. Hit thresholds can be set to compare pre- vs. post-compound scans and positive hits are displayed in “yellow”. The hit threshold in this experiment displays post-compound reads that are less than 80% of the current magnitude of the pre-compound read.

hERG single point screening run (6 compound plates)

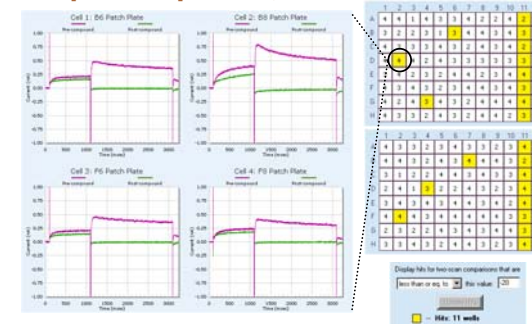


Fig 6. **Screen displays of raw data and compound plate view.** Raw data traces of pre- and post compound hERG currents (left). The four sets of traces are the four PatchPlate wells where dofetilide was added from position D2 on the compound plate (upper right). A second compound plate from the same screen is also shown (mid. right). The hit configuration is shown to display in yellow the wells on the compound plate that had an average current magnitude drop in the post-/pre-currents of 20% or greater.

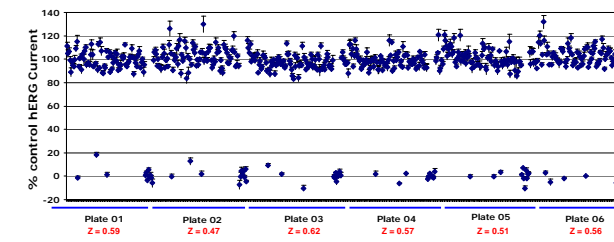


Fig 7. **Summarized data for a 6 plate (480 compound) screen.** Data from the six individual runs are shown with corresponding z-factor.

IC₅₀ curves for the “hit” compounds

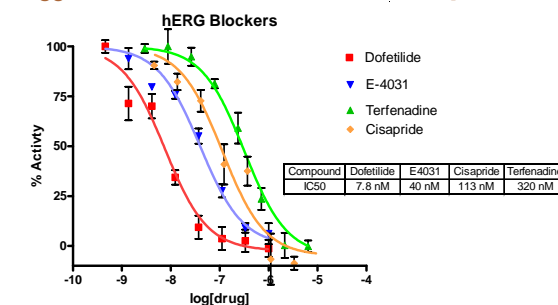


Fig 8. **IC₅₀ curves from single 45 minute runs.** IC₅₀ curves for dofetilide, terfenadine, and E-4031. Each curve is obtained from a single run using 3 columns on a 96 well plate (n ≤ 12 cells/concentration).

hERG performance trendline

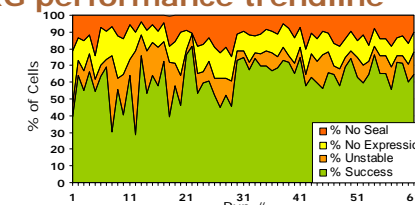


Fig 9. **Trend graph for a hERG cell line.** Sixty-one runs are shown here for hERG expressed in CHO cells. This graph represents over 14,000 successful recordings.

Na_v 1.5 currents measured on the IonWorks HT

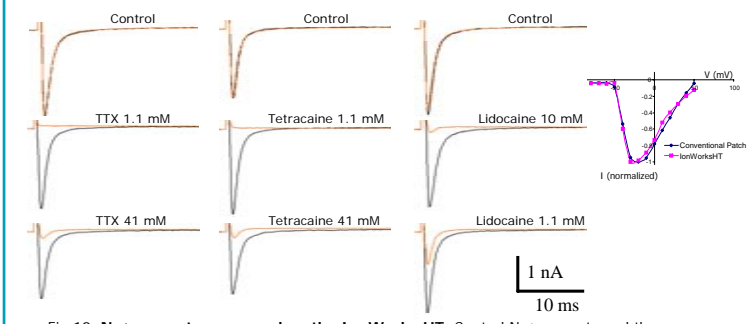


Fig 10. **Na⁺ currents measured on the IonWorks HT.** Control Na⁺ currents and those measured in the presence of “mock” compound TTX and tetracaine (left). Superimposed I/V curves (right) from cells measured with conventional patch clamp methods and with the IonWorks HT. In both cases the peak currents were near 3 nA.

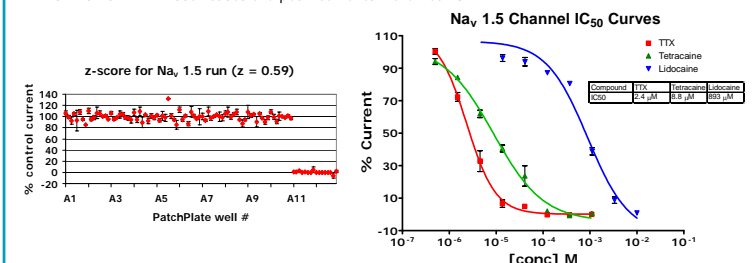


Fig 11. **Na⁺ current z-score and pharmacology on the IonWorks HT.** Z-score calculation from a single run (231 cells) on the IonWorks HT (left). IC₅₀ curves for TTX, Tetracaine and Lidocaine measured on the IonWorks HT (right). Each curve is obtained from a single experimental run using 3 columns of a 96 well compound plate (n ≤ 12 cells/concentration).

IonWorks™ HT instrument summary and details:

- ◆ Instruments in place at both Pharma and Biotech companies
 - Some customers have multiple units
 - Used for pharmacology, safety profiling, and hit confirmation
- ◆ Features
 - High throughput – up to 3000 patches/day
 - 90–180 cmpds./day (n=4/concentration, 5-10 point IC₅₀ curves)
 - > 250,000 recordings performed at MDC in the last 10 months at a 60 – 85% success rate
- ◆ Validated with a broad range of channels
 - Kv 1.5, hERG, Nav (1.5,1.3), Ca²⁺ channels (L, N, and R-type)
- ◆ Validated with a broad range of cell lines
 - CHO-K1, HEK 293, Rin-5F, Neuroblastoma, primary neurons
- ◆ Intellectual property position: 2 foundation patents (US 6,488,829 and EP1040349) plus numerous other patents pending

Acknowledgements:

We would like to thank Cytomx (Cambridge, UK) for providing the CHO hERG cells and Roche for providing the CHL Nav 1.5 cells.