

Unattended Screening Workflow in a 384-well Automated Patch Clamp System

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Introduction

Within ion channel working group efforts (Fermi *et al.*, 2015), reference compounds were tested across multiple sites and automated patch clamp (APC) platforms (Kramer *et al.*, 2020). Resulting IC₅₀ values were variable between drugs and platforms. In general, as Iversen *et al.* (2012) outlined, the quality of an assay is defined by the robustness and reproducibility of the measurement signal in the absence of any test compounds. Data robustness and reliability using an APC instrumentation should be proven carefully before an unattended full-day screening workflow is implemented.

In this study, our goal was to outline an APC recording routine to investigate compounds in an automated and unattended mode for a full working day.

After compound preparation, the experimental run was executed and 4-point dose-response curves of amitriptyline, bepridil, chlorpromazine, clozapine, flecainide, quinidine and verapamil were recorded on Nav 1.5 cells. Positive and negative controls were included to calculate Z' values. At the beginning and at the end of the unattended run period control plates containing only 0.3% DMSO controls instead of compounds were measure to determine a false positive hit rate.

In a 6 hour measurement period, 10 NPC-384 patch clamp chips were measured with n = 6 replicates per compound concentration. Z' values of 0.91 ± 0.004 for the 1st peak and 0.89 ± 0.006 for the 2nd peak (inactivated state) prove low variability of the data. The average rundown of 0.3% DMSO control solution was found to be 4.4% ± 0.5% for the peak 1 and 5.5% ± 0.9% for the peak 2 (n=374 wells across 10 plates). Success rates for completed experiments were in excess of 94% for every plate.

Our statistical measures of assay performance over a typical full working day in a HTS environment showed that the 384-well automated patch clamp system used in our study (SyncroPatch 384i) delivers high reproducibility of IC₅₀ values across the same chip and across experiments.

SyncroPatch 384i unattended screening

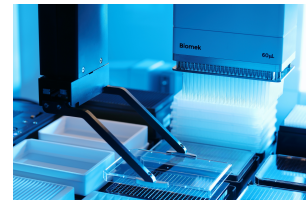


Figure 1 The SyncroPatch 384i robotic system is based on the Biomek i5 state-of-the-art liquid handler.

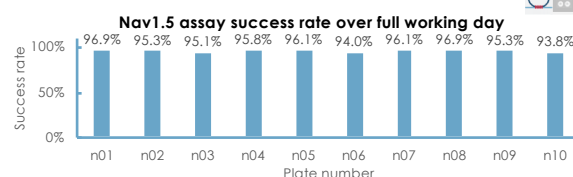


Figure 2 Success rate over time (# of plates used within 1 working day) of Nav1.5 overexpressing CHO cells. QC criteria were 200 MΩ/cell, current amplitude 200 pA. Recordings were performed in perforated patch using multihole patch clamp plates (4 holes per well).

Nav1.5 – Two-pulse protocol (Peak1, 2)

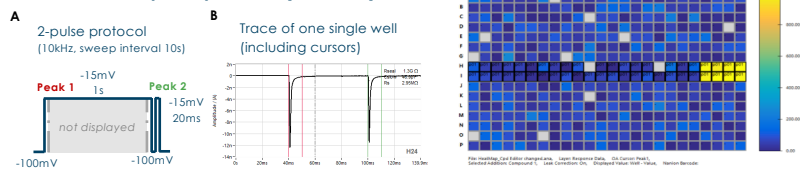


Figure 3 **A** Two-pulse protocol to investigate Nav1.5 blockers and their state dependency. **B** Exemplary traces of Nav1.5. **C** Heat Map display of a 384-well plate including besides 8 wells of positive control (50 μM Tetracaine, yellow) only negative control wells (0.3% DMSO). The facets of blue are representing a good current stability over the recording time (10 min).

Nav1.5 – rundown and false positives

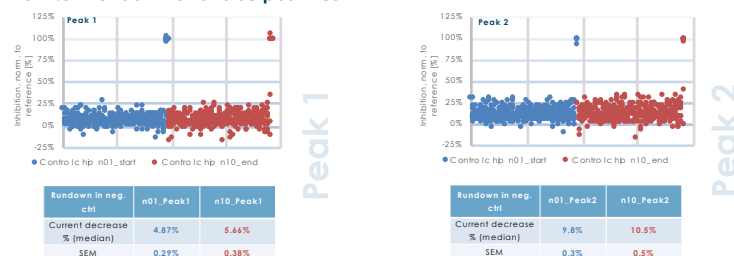


Figure 4 Percent decrease of Nav1.5 (left: peak 1, right: peak 2) current amplitude in control plates at the beginning and at the end of the unattended run over 10 x 384-well recording plates. Plates contained 0.3% DMSO (376 wells) and a full block concentration of 50 μM Tetracaine (8 wells).



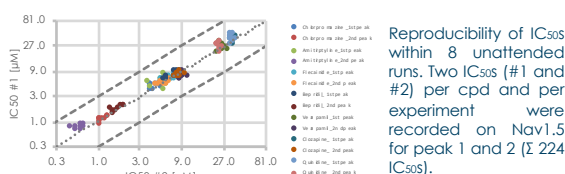
Figure 5 False positives, calculated for peak 1 and 2. Tables show number of wells and hereof calculated percentage of false positive hits at cutoff at 20% and 25% block. Blue: values at beginning and, red, at the end of the 6 h run.

SyncroPatch 384i Z prime and reliability of results

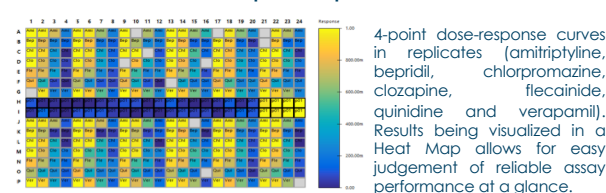
The platform allows for unattended runs in a fully automated manner. Independent movements of gripper and pipette head and a selective tip option for serial compound dilution enable a workflow that fits an HTS screening environment.

Compound	Min IC ₅₀ (μM)	Max IC ₅₀ (μM)	Ratio max/min	Average IC ₅₀ (μM)	SEM	Plate	Z' (Peak1)	Z' (Peak2)
Amitriptyline_1	3.83	5.98	1.65	5.16	0.31	n01	0.91	0.89
Amitriptyline_2	4.30	7.84	1.82	5.42	1.03	n02	0.90	0.89
Bepridil_1	5.82	8.39	1.44	7.18	0.98	n03	0.91	0.88
Bepridil_2	6.34	9.63	1.52	7.93	1.27	n04	0.91	0.90
Chlorpromazine_1	3.93	5.42	1.38	4.51	0.54	n05	0.89	0.88
Chlorpromazine_2	4.27	5.67	1.33	4.94	0.46	n06	0.92	0.91
Clozapine_1	33.5	36.6	1.09	34.9	1.04	n07	0.91	0.91
Clozapine_2	30.0	39.8	1.33	35.9	2.86	n08	0.91	0.90
Flecainide_1	5.42	7.70	1.42	6.64	0.69	n09	0.93	0.93
Flecainide_2	6.04	7.84	1.30	6.66	0.54	n10	0.88	0.87
Quinidine_1	31.6	37.7	1.19	33.7	1.81			
Quinidine_2	28.5	48.2	1.69	38.9	6.33			
Verapamil_1	23.6	30.0	1.27	26.2	2.02			
Verapamil_2	21.5	31.3	1.46	26.0	3.16			
Median Z'							0.91	0.89
SEM							0.004	0.006

Figure 6 Variance across experiments. The left table displays the min and max IC₅₀ values of 14 compounds on 8 plates (each cpd was tested twice per plate). The right table shows the Z' values for each experiment considering 2 rows of control values per plate.



Heat Map of compound results



References

- Fermi B *et al.*, (2015) A New Perspective in the Field of Cardiac Safety Testing through the Comprehensive In Vitro Proarrhythmia Assay Paradigm. *J Biomol Screen*, 21(11):1-11, doi: 10.1177/1087057115594589.
 Kramer *et al.*, (2020) Cross-site and cross-platform variability of automated patch clamp assessments of drug effects on human cardiac currents in recombinant cells. *Sci Rep* 10, 5627 (2020). <https://doi.org/10.1038/s41598-020-62344-w>
 Iversen, PW *et al.*, (2012) HTS assay validation 2. Stability and process studies. In G. Sittampalam, A. Grossman, K. Brimacombe, & E. Al (Eds.). *Assay Guidance Manual*. Eli Lilly & Company and the National Center for Advancing Translational Sciences.