Poster #051-A

Assessing Variability of hERG Data Generated Using a Mock Action Potential Waveform and Automated Patch Clamp Platforms – A HESI-Coordinated, Multi-Laboratory Comparison of 28 Drugs Across 3 Platforms

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Background

hERG block is the most common mechanism of drug-induced QT_C prolongation and the potentially fatal ventricular arrhythmia Torsade de Pointes. Assessing hERG block is thus important to understand cardiac safety and support First-in-Human studies. The new ICH E14 Q&As 5.1 and 6.1 further describe how hERG data generated following ICH S7B Q&A 2.1 best practices, coupled with in vivo QT results, can support clinical interpretation of QT studies as a part of an integrated proarrhythmic risk assessment. The evolving regulatory landscape illustrates how knowledge gained regarding the cellular mechanisms of clinical risk could lead to increasing use of nonclinical strategies to support clinical decision-making.

Nearly all hERG assays submitted to the FDA have been obtained by electrophysiology experts operating the manual patch clamp (MPC) systems – a technically challenging and labor-intensive method. In contrast, automated patch clamp (APC) platforms remove technical barriers to generate ion channel data and offer high-throughput capabilities. These advantages are achieved by adjusting experimental conditions such as using fluoride (F^-) to reduce artificial leak current, fixed duration recordings across all cells, and the use of cell population recordings with multi-hole chips to optimize signal-to-noise ratio and frequency of catching cells. APC platforms are routinely used by industry to screen cardiac ion channel liability to aid compound selection. Nonetheless, there remains concerns regarding the impact of altered experimental conditions in APC (relative to MPC) and data quality derived from these platforms.

Several studies have examined variability in APC data (Kramer et al., 2020; Elkins et a., 2013; Watts et al., 2022). Kramer et al. evaluated 12 drugs on four cardiac ionic currents across five platforms by 17 labs. Each drug on each current was evaluated once by each lab. The results showed that the difference in block potencies varied by drug, ionic current, and APC platform, leading to the recommendations to ascertain exposure, achieve steady state inhibition, isolate current-of-interest, and synchronize experimental conditions/protocols to reduce variability. Elkins et al. and Watt et al. performed retrospective analyses of cardiac ion channel data obtained repeatedly to demonstrate distribution of block potencies. These results have led to the recommendation of repeating assays by Elkins et al. to reduce uncertainty.

Understanding APC data reproducibility is a basic step toward applying these data for regulatory decision-making. The present study – a part of a HESI-coordinated international ion channel research effort – continues to build upon the current understanding. Four participating labs tested 14 to 28 drugs on four cardiac ionic currents using three different APC systems. This poster focuses on the hERG results.

Methods

Whole cell patch clamp experiments were conducted to obtain block potencies. Each lab used its own cell lines. **Table 1** summarizes the recording temperature, platform, recording configuration, # of drug applications (to try to reduce the extent of nonspecific binding-related drug loss), and major anion in internal solution used to obtain hERG data.

Lab/ Recording Temperature	Metrion Biosciences (ambient)	Nanion GmbH (25-27°C)	Nanion GmbH (35-37°C)	Charles River Laboratories (CRL UK) (ambient)	Eurofins Discovery NA (24-27°C)	Eurofins Discovery NA (36-37°C)
Platform	QPatch 48	SyncroPatch 384i	SyncroPatch 384i	Qube 384	Qube 384	Qube 384
Recording configuration	Single hole	4-hole	Single hole	Single hole	10-hole	10-hole
# of drug applications	4-8X	1X	1X	3X	2X	2X
F^-:Cl^- (mM)	60 KF: 60 KCl	60 KF: 60 KCl	60 KF: 60 KCl	70 KF: 50 KCl	120 KF: 20 KCl	120 KF: 20 KCl

Table 1. Single hole means recordings were obtained from individual cells; 4- and 10-holes means population recordings from up to 4 and 10 cells, respectively. Ambient temperature means temperature was not controlled or monitored. F^-:Cl^- refers to fluoride:chloride concentrations in mM.

The voltage command mimics a ventricular action potential and was presented at 0.2 Hz (https://www.fda.gov/media/151418/download). Overall variability of the hERG data was estimated using the metafor package in R (Viechtbauer 2010).

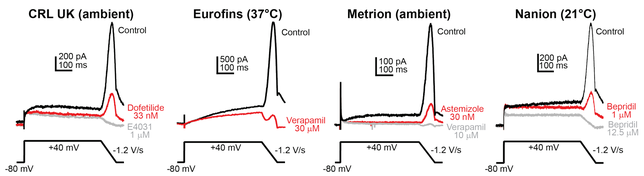

Figure 1. Representative hERG current traces/recordings obtained by each APC lab. The prepulse to -90 mV to assess input resistance is removed to focus on the hERG current.

Results

Data from experiments that did not achieve 50% current inhibition by the highest tested concentration were excluded from analyses (n=11).

No systematic differences were observed amongst hERG data generated across all drugs at ambient temperature or RT by different labs (**Figure 2**).

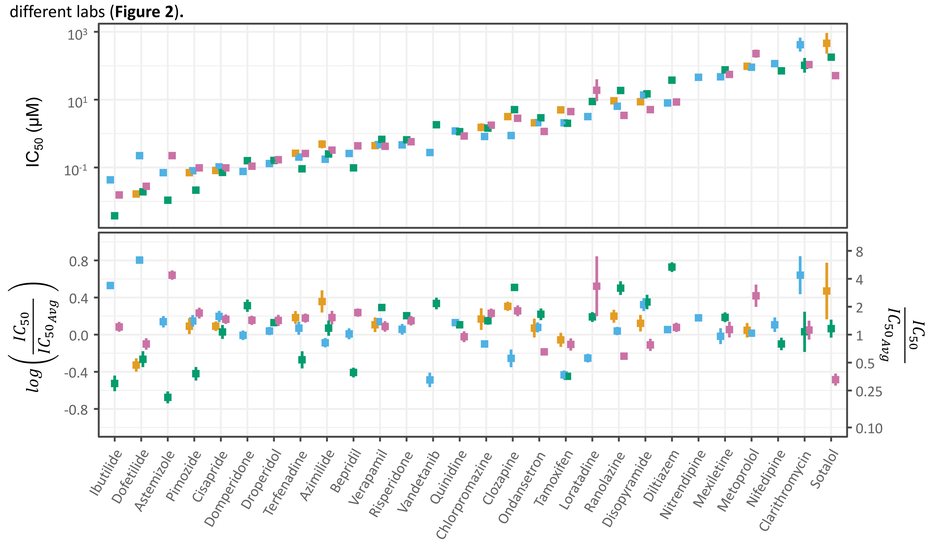

Figure 2. Top panel, IC_{50} values for the studied drugs at ambient temperature or RT. Each color represent a different lab: CRL UK, Eurofins, Metrion, and Nanion. The drugs are organized by their average IC_{50} values across all laboratories. Error bars represent the 95% confidence interval (CI) for the IC_{50} values. In some instances, the error bars are obscured by the symbols. Bottom panel, pIC_{50} (or -log(IC_{50} [M])) with average drug pIC_{50} subtracted. The left y-axis represents difference in pIC_{50} whereas the right y-axis represents the fold difference of individual data-points to the group average.

Two labs also acquired data at near PT (**Table 1**). Higher block potencies were observed at PT in 26 out of 27 drugs tested by Eurofins and in 23 out of 25 drugs tested by Nanion.

On average, the hERG block potencies were systematically higher (1.7-fold) at near PT compared to RT (**Figure 3**).

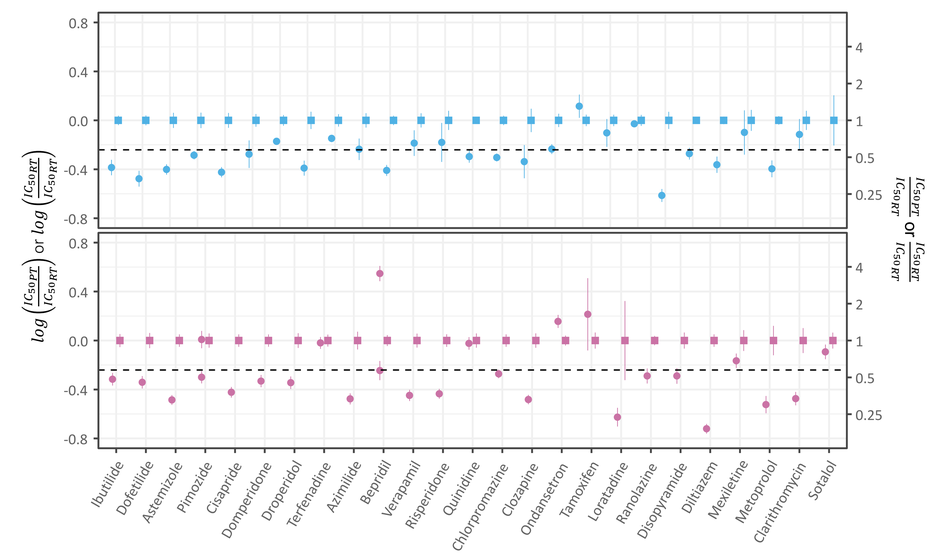

Figure 3. Two labs tested drugs at near physiological temperature (PT) and RT/ambient (top: Eurofins; bottom: Nanion). The figure shows the differences between pIC_{50} at two temperatures. ● and ■ symbols reflect near PT and RT, respectively. Note that all data were used in the analysis. However, the figure excludes two data points due to a substantial difference for each laboratory.

References

Kramer J, et al. Sci Rep 2020;10(1):5627; Elkins RC, et al. J Pharmacol Toxicol Methods 2013;68(1):112-22;

Watt ED, et al. J Pharmacol Toxicol Methods 2022;118; Viechtbauer W. J Stat Softw 2010;36(3):1-48

The overall variability was estimated as 16x using random-effect meta-analysis accounting for drug- and lab-specific effects (**Figure 3**). For the two labs that collected data at two temperatures, the variability was numerically higher at PT compared RT. The overall variability with APC is larger than the variability that as observed with manual patch clamp (~5x) (Poster #035-A). Kramer et al. used "repeatability coefficient" ($\sqrt{2} * 1.96 * \varepsilon$) to describe variability associated with different APC platforms. This metric reflects the smallest detectable difference and was estimated to range from 3.7 to 9.7 for different platforms compared to 7.2 in the present study.

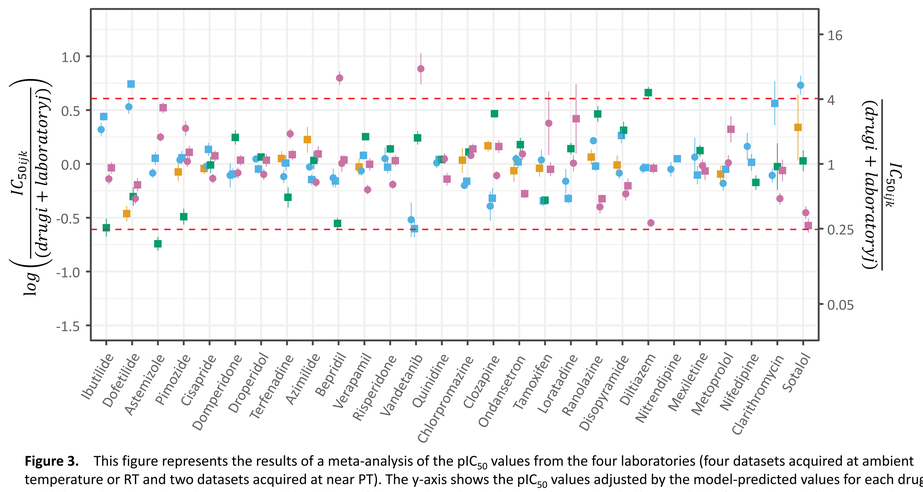

Figure 3. This figure represents the results of a meta-analysis of the pIC_{50} values from the four laboratories (four datasets acquired at ambient temperature or RT and two datasets acquired at near PT). The y-axis shows the pIC_{50} values adjusted by the model-predicted values for each drug and lab combination. Error bars represent the 95% confidence intervals. Dashed red lines indicate the variability factor derived from the meta-analysis model. This analysis aggregates data to provide an estimate of variability in hERG block potency.

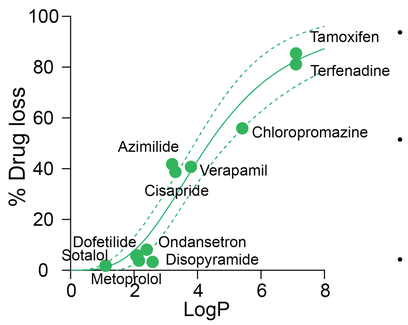

Figure 4. Percent drug loss vs. LogP. Drug solutions were prepared in glass insert vials in a microtitre plate for loading into QPatch. Measured drug concentrations from samples collected from these vials were compared with measured drug concentrations from samples collected from the waste reservoir after application to Qplate. Drug concentrations were measured using LC-MS/MS.

- Nanion and Metrion collected drug samples in satellite experiments for concentration verification.
- Two methods were used to collect and store drug samples (per design of each APC platform):
  - Nanion: automated sample collection into 96 well plates
  - Metrion: manual sample collection into polypropylene tubes
- Bioanalytical challenges were encountered with simultaneous analysis of numerous drugs in the same 96-well plate (i.e., differences in drugs stability (bench top and freeze/thaw stability), differences in sample processing and LC-MS/MS methods required running multiple instruments with careful coordinating efforts).
- Even with 4-8X drug applications to reduce nonspecific binding, drug loss was still observed and the extent of which positively related to LogP. This is consistent with HESI BAA MPC experience.

Conclusions / Discussion Topics

- HESI BAA APC effort is almost complete, with two labs continuing with $Ca_V1.2$ data collection.
- No systematic differences in the results were observed amongst the participating APC labs.
- Drug potencies collected at near PT are systematically higher than RT by two labs (but also see Poster #047-A).
- Variability of APC is larger than MPC and is within the range of repeatability coefficients reported by Kramer et al.
  - Additional data analysis will focus on understanding the impact of data acceptance criteria by each lab to understand their impact on drug block potencies.
  - Sample collection and concentration verification schemes to support assessing drug loss for APC with high throughput platforms require collaboration amongst platform providers, APC operators, and bioanalytical experts handling the samples.
- Results of this study will inform the expected variability of hERG data under best practice recommendations feasible/practical for APC platforms and using these data to support integrated nonclinical risk assessment.

Learn more about HESI Cardiac Safety

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