>> Standards for hiPSC-derived cardiomyocyte electrophysiology using the MEA assay

Mike Clements[1,✉], Cristina Altrocchi[2], Devon Guerrelli[3], Adam P. Hill[4], Nikki Posnack[3], Chris Strock[5], & Takashi Yoshinaga[6]
[1]Axion BioSystems, GA, USA; [2]Johnson & Johnson, Belgium; [3]Children's National Hospital, DC, USA; [4]Victor Chang Cardiac Research Institute, Australia; [5]Cyprotex, MA, USA; [6]Eisai Co., Ltd, Japan. ✉ Email: mclements@axionbio.com

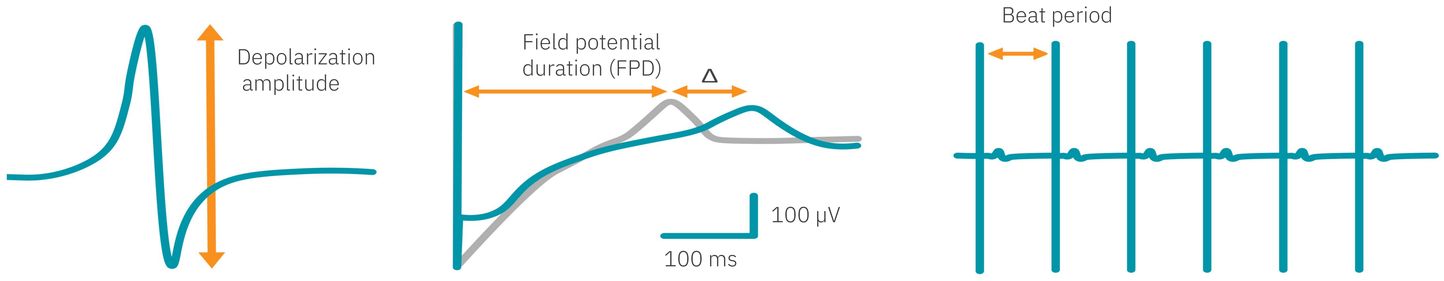
Advancing hiPSC-CM assay adoption will require standardization for data comparison and validation

Background

Cardiac stem cell models are transforming research and discovery—but a **lack of standardized criteria** to assess functional activity can lead to inconsistent results. Over the last decade, the multielectrode array (MEA) assay has become a popular tool for characterizing hiPSC-cardiomyocyte (CM) batches, studying disease models, screening therapeutics, and evaluating drug-induced cardiotoxicity.

The goal of this project is to **set the minimum acceptance criteria** for a spontaneous beating wild type hiPSC-ventricular cardiomyocyte field potential assay **for compound testing and/or disease modeling**.

Identifying key cardiac metrics



Leveraging in-house experience in academia and industry, published data, and international consortia (CiPA and JiCSA) validating hiPSC-cardiomyocyte assays, we have developed the **Axion iPSC Model Standards (AIMS)** framework. This proposed standard focuses on the **spontaneous beat rate**, features of the cardiac waveform (**depolarization spike amplitude** and **field potential duration**), and the synchronization of activity in the syncytia.

AIMS #CM01: Defining minimal acceptance criteria for wild-type hiPSC-ventricular cardiomyocyte

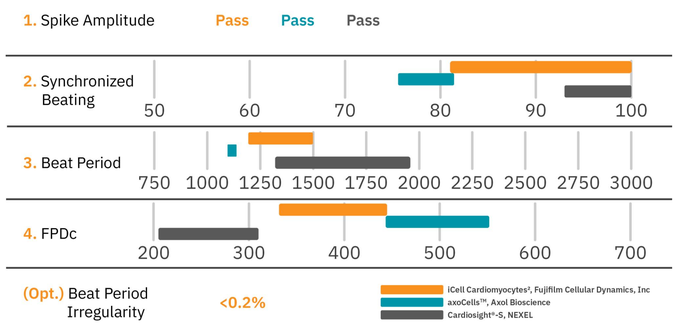
AIMS #CM01: Proposed standards

An hiPSC-CM cell source is considered to meet standards when **≥80% of MEA wells** meet all four of the following well acceptance criteria:

1. Spike amplitude of **≥0.5 mV** on ≥50% electrodes .
2. Synchronized beating across **≥50% electrodes**.
3. Spontaneous beat period of **750-3000 ms** (i.e. 20-80 beats per minute, BPM).
4. Corrected field potential duration (FPDc) of **200-700 ms**. Fridericia correction: FPDc = FPD/(Beat Period)$^{1/3}$

(Optional) Beat period irregularity **<0.2%**

Standards accommodate a range of cell sources



This figure shows three vendor sources where the specification was met. Specification range was inferred from Millard et. al. 2018[1]. Although basal activity of vendor cell sources differed significantly, **consistent concentration-dependent effects were observed[1].**

Important considerations

- Reproducibility and quality is dependent on culture protocols. Participating vendor protocols must specify key details (see AIMS website).
- The synchronized beating should initiate from a single point of origin. Competing pacemakers can confound analysis
- For compound-induced effects on spike amplitude, ≥1.0 mV would provide a larger assay window.
- Primary ventricular CMs do not spontaneously beat, and this behavior in iPSC-CMs is often attributed to "immaturity." As models develop, a paced hiPSC-CM assay could be the subject of a future AIMS but is beyond the scope of this AIMS.

Select References

1. *Millard D et al. Cross-Site Reliability of Human Induced Pluripotent stem cell-derived Cardiomyocyte Based Safety Assays Using Microelectrode Arrays: Results from a Blinded CiPA Pilot Study. Toxicol Sci. 2018 Aug 1;164(2):550-562.*
2. *Blinova K et al. International Multisite Study of Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes for Drug Proarrhythmic Potential Assessment. Cell Rep. 2018 Sep 25*
3. *Cooper B et al. Comparative cardiotoxicity assessment of bisphenol chemicals and estradiol using human induced pluripotent stem cell-derived cardiomyocytes, Toxicol Sci, 2024 Apr 2;198:273-287*
4. *Thorpe, J et al. Development of a robust induced pluripotent stem cell atrial cardiomyocyte differentiation protocol to model atrial arrhythmia. Stem Cell Res Ther 14, 183 (2023).*
5. *Clements M et Al. High-Throughput Multi-Parameter Profiling of Electrophysiological Drug Effects in Human Embryonic Stem Cell Derived Cardiomyocytes using Multi-Electrode Arrays. Toxicol Sci. 2014 Aug 1;140(2):445-61.*

More references available at www.axionbiosystems.com/axion-ipsc-model-standards-aims/aims-cm01

Acknowledgements

Thanks to FCDI, Axol Biosciences, and NEXEL for sharing their hiPSC-cardiomyocyte electrophysiological characterization data.

Additional thanks to Daniel Millard, Stacie Chvatal, Anthony Nicolini, Parker Ellingson, and Rika Yamazaki for AIMS review.

Learn more about AIMS

Scan the QR code to visit our website for more information about AIMS, iPSC model standardization, and provide feedback.

Presented at ISSCR 2025, Hong Kong. Poster Number: W1284. June 11, 2025