

# Calcium Transient Assay for Cardiac Arrhythmic Potential Evaluation: using human iPS-Derived Cardio Myocytes

**Commonly used acronym:** CTCM on hiPS-CMs

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## Organisation

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**Country** Belgium

**Geographical Area** Flemish Region

## SCOPE OF THE METHOD

|   |   |
|---|---|
| <b>The Method relates to</b>                    | Human health, Other   |
| <b>The Method is situated in</b>                | Regulatory use - Routine production, Translational - Applied Research, Other: Safety Pharmacology |
| <b>Type of method</b>                           | In vitro - Ex vivo  |
| <b>Specify the type of cells/tissues/organs</b> | hiPSC-derived cardiomyocytes  |

## DESCRIPTION

### Method keywords

Calcium transient

Proarrhythmia

Stem cell

Fluorescent Dye Calcium-sensitive Imaging

Cardiomyocyte

hiPS-CMs

Human induced Pluripotent Stem Cell

### Scientific area keywords

Safety Pharmacology

Side effects

medium-high throughput assay

cardiovascular derisking

regulatory

### Method description

Cell Culture and Reagents hiPSC-CMs can be obtained commercially either as living pre-plated cells seeded onto fibronectin-coated 96-well mClear plates (Greiner Bio-One, No. 655090) or can be plated in house at a density (~25,000 cells/well) suited to forming a confluent synchronously beating monolayer. Most commercially offered cardiomyocyte lines represent a mix of ventricular, atrial and nodal cells and derived from one human donor. Cells were cultured with commercial culture medium (optimized for the specific cell line) in a humidified incubator at 37°C and 5% CO<sub>2</sub>, with medium being changed once a day. On the day of the experiment, the culture medium was replaced with Tyrode's solution (Sigma, No. T2397) supplemented with 10 mM HEPES together with KCl to represent isokalemic (4.2 mM K<sup>+</sup>) conditions. As a calcium-sensitive fluorescence dye Cal-520 AM (Cat. No. 36,338; AAT Bioquest) was used to capture the intracellular calcium transients. Accordingly, Cal-520 was incubated for 45 min followed by a washout and a 30-min recovery before starting the

experiments. Calcium Transient Measurements Spontaneous beating activity of hiPSC-CMs was assessed through measurement of the  $\text{Ca}^{2+}$  fluorescence signal integrated over the whole well. Fluorescence signals were measured using the Hamamatsu FDSS/mCell platform and the records subsequently analyzed offline using NOTOCORD-hem software (version 4.3), containing EXT modules and an algorithm developed by XiTechniX to detect beat-by-beat Amp, BR, and CTD90 parameters. All wells within a plate were measured simultaneously using the following FDSS/mCell settings: sampling frequency 66.7 Hz, exposure time 14.6 ms, excitation wavelength 480 nm, emission wavelength 540 nm, temperature controlled at 37°C. First, the experimental plates were put into the FDSS/mCell to stabilize for 10 min. Next, a baseline recording was run for 3 min followed by compound addition. The effect of a compound was recorded (5-min recording time) around 15 and 30 min after compound addition. CTD90, BR, and Amp were quantified for baseline and 30-min compound effects as the median value of all beats (calcium transients) measured within a 1-min interval of the recording. The recording around 15 min was used only for observation of Early-After-Depolarisation (EADs) or fibrillation-like events. EADs were manually monitored and evaluated. Cessation of beating was defined after 30 min in case BR was <5 beats/min. Wells that temporarily stopped beating during compound addition but recovered at the 30-min time point were not defined as beat stop.

## **Lab equipment**

Laminar flow hood ;

Cell Incubator ;

Hamamatsu FDSS/mCell platform ;

Analysis software.

## **Method status**

Internally validated

Published in peer reviewed journal

Validated by an external party (e.g. OECD, EURL ECVAM,...)

## **PROS, CONS & FUTURE POTENTIAL**

### **Advantages**

Early (fast) evaluation of arrhythmic potential using human cells

## Challenges

Maturation of cells remains debate ;

Cell-layer don't reflect 3D-complexity of a (human) heart ;

Commercial cell line represents  $n = 1$ .

## Modifications

Use of more mature cells (due to improved culture conditions ?) ;

Improved phenotypic appearance (obtaining closer cellular resemblance of the human heart).

## Future & Other applications

Calcium transient method can potentially be applied for measuring activity of other cell types as well

## REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

### References

Lu HR et al., Assessing Drug-Induced Long QT and Proarrhythmic Risk Using Human Stem-Cell-Derived Cardiomyocytes in a  $\text{Ca}^{2+}$  Imaging Assay: Evaluation of 28 CiPA Compounds at Three Test Sites. Toxicol Sci. 2019 Aug 1;170

Kopljär I. et al., Development of a Human iPSC Cardiomyocyte-Based Scoring System for Cardiac Hazard Identification in Early Drug Safety De-risking. Stem Cell Reports. 2018 Dec 11;11(6):1365-1377

Kopljär I. et al., Impact of calcium-sensitive dyes on the beating properties and pharmacological responses of human iPS-derived cardiomyocytes using the calcium transient assay. J Pharmacol Toxicol Methods. 2018 May - Jun; 91: 80-86.

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