Improved Sustainability Through the Use of Assay Ready Cells to Support Ion Channel Screening

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a better decision

Each year the consumables and equipment required for day-to-day cell culture generates tons of plastic waste, which cannot be reused or recycled. This results in a significant impact to the environment and increases the cost of running a lab [1]. Our work in ion channel screening means we rely heavily on single-use plastics which carry a financial and environmental cost and contribute to our carbon footprint. It has been estimated that each month our cell culture department generates an average of 80kg of consumable and plastic waste. In addition, recycling plants won't accept lab waste due to contamination.

One solution is to use assay ready cells in place of continuous cell culture. These cells are frozen and stored at high density and thawed immediately prior to assay, eliminating the need for prolonged culture. In this study we generated assay ready cell lines expressing a number of different ion channels and tested their performance on automated patch-clamp platforms. These cells showed excellent viability and functional activity, comparable to live cultured cells. In addition, the use of assay ready cells on automated patch-clamp systems has allowed us to streamline laboratory processes, halving the time it would usually take to generate client drug safety data. These steps have minimised the amount of plastic waste generated from cell culture by ~50% over the last 6 months. Extended testing is ongoing to confirm the reproducibility of these highly functional assay ready cell lines, and to implement these methods across all cell lines used at ApconiX.

AIMS AND METHODS

To implement this technique into our lab and quantify benefits in terms of cell functionality comparable to live cells, we aimed to produce assay ready cells which:

- Are frozen at high viability, making them fit for drug safety screening and can be used in patch-clamp assays directly after thawing without culture or passage [2].
- Express functional ion channels of NaV1.5, Ito, Kir2.1 and hERG in assay-ready aliquots of 10million cells/ml that provide a stable membrane seal and display strong, consistent currents.

Cell Treatment - Cell lines were expanded as usual for approx. 2 weeks prior to cryopreservation and lifted using Accutase from adherent culture flasks.

- Cells were frozen at a controlled rate courtesy of Cryoniss Ltd. at a density of 10million cells/ml in 90% freezing media and 10% DMSO to -80 before transfer to liquid nitrogen. (CHO Freezing Media; F12 + 10%FBS +P/S +NEAA. HEK Freezing Media; DMEM + 10%FBS +P/S +NEAA +L-glutamine)
- Cells thawed in 37˚C water bath for 2minutes and recovered in thawing media, spun and resuspended in assay buffer ready for use.

Several freezing/thawing treatment methods were tested, however made little difference to overall cell health and performance for automated patch. The most time efficient and logical method of thaw straight into media/assay buffer was chosen.

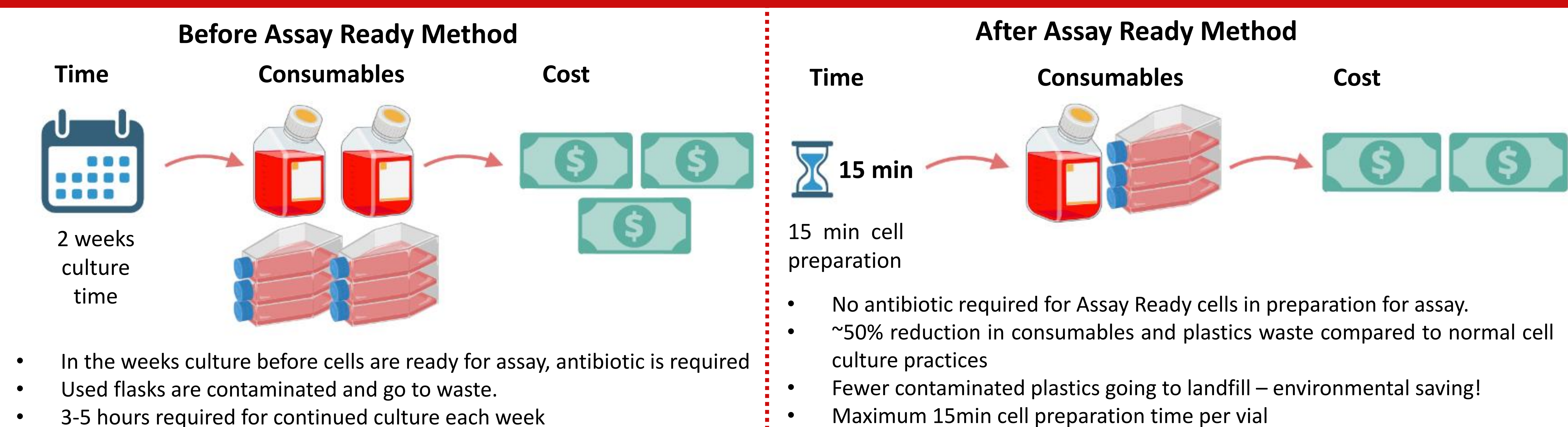
Assay conditions - External buffer for each assay was (in mM): NaCl 140, KCl 4, MgCl2 1, CaCl2 2, HEPES 10, Glucose 5.

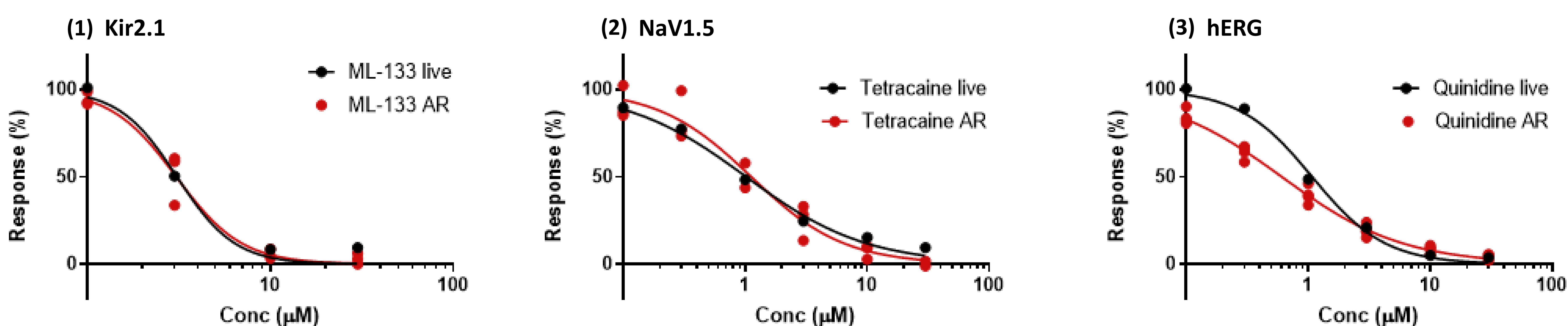
Kir2.1 Experiments were performed on Patchliner (Nanion Technologies) Internal buffer (mM): NaCl 10, KCl 50, HEPES 10, KF 60, EGTA 20, NaATP 4. Voltage protocol: -90mV holding potential, step to -140mV for 100ms, ramp to +50mV for 500ms.

hERG Experiments were performed on QPatch II (Sophion Bioscience) Internal buffer in mM (hERG): KCl 120, MgCl2 1.75, CaCl2 5.3, HEPES 10, EGTA 10, NaATP 4. Voltage protocol: -90mV holding potential, step to +40mV for 1000ms, step to -40mV for 1000ms.

NaV1.5 Experiments were performed on QPatch II. Internal buffer (in mM): NaCl 10, HEPES 10, CsF 130, EGTA 1. Voltage protocol: -90mV holding potential, step to 0mV for 20ms.

APPLICATION OF ASSAY READY CELLS AND RESULTS





The concentration-response curves of assay ready NaV1.5, hERG and Kir2.1 correlated with that of live cultured cells (1, 2 and 3). Assay ready NaV1.5 had high success rates (>93% efficiency) comparable to live NaV1.5 cells (Avg. 92.2%). Analysis of hERG and Ito assay ready cell lines showed similar success rates (91% Average for each) reached across the cell library.

DISCUSSION AND CONCLUSIONS

Implementing the use of assay ready cells for automated patch clamp has:

- Made sustainability an integral part of everyday lab work and reduced our plastic consumables waste, ensuring an environmental saving.
- Yielded success rates which are at least as good as live cells from a growing culture.
- Saved time spent in cell culture improving laboratory efficiency and turnaround time for client data, in the long-term saving our client's time.

REFERENCES

1. Urbina, M., Watts, A. & Reardon, E. (2015). Labs should cut plastic waste too. *Nature* **528**, 479.
2. Wehmeier, O., & Loa, A. (2020). Turning Cells into Reagents: The Preparation of Assay Ready Cells for Routine Use in Bioassays. *Methods in molecular biology (Clifton, N.J.)*, *2095*, 17–25.