# Reducing plate edge effect by controlling cell handling conditions for *in vitro* tumor hypoxia assays

Poster #2438

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## Abstract

**To reduce the impact of high variability in edge wells of 96-well plates, the "edge effect", researchers often fill edge wells with buffer to try to insulate the rest of the plate. This removes over a third of the wells from the assay, adding cost, handling time, and space problems to *in vitro* assays. This is of particular concern for researchers growing cells in low oxygen, as handling more plates increases the time that cells are out of optimum conditions, risking HIF-1a modulation. For this project, we tested the idea that controlling the entire cell handling space to conditions that match the incubator, including working space floor temperature and gas levels, may help reduce the edge effect seen in 96-well plates. The null hypothesis was that cell handling conditions would make no difference in the variability of cell density in 96-well plates loaded in traditional room air conditions compared to plates loaded in controlled conditions matching the incubator. To address cell loading, specifically, A549 cells were harvested from cell culture flasks in traditional cell handling conditions (room temp, 20% $O_2$, 0%$CO_2$), then split. Half of the cells were transferred to an Xvivo System for controlled cell handling conditions. The cells in both conditions were transferred to media pre-conditioned to hypoxic incubator conditions and then plated in 96-well plates. Temperature mapping was performed with 15 thermal probes simultaneously in replicate plates loaded with medium only. The cells were incubated for 24 hours, then stained for cell density, and read on a plate reader. Cell viability was greater than 90% (n=4 plates/condition/trial x 4 trials). We found that edge well temperatures were more stable in plates handled in unbroken optimal conditions and that edge effect on cell density was greatly reduced. In room temperature conditions, columns 1 and 12 cooled most rapidly. We concluded that full-time control of environmental conditions allowed for use of the whole plate with reduced edge effect. This helps reduce cost, time, # of plates, and the risks to cells during cell handling.**

## Background

- **Edge effect in 96-well plates has been recognized since their early use in the 1970s [1]**
- **Thermal gradients in edge wells disrupt even cell settling over time [2]**
- **Techniques such as leaving the cells in the room temperature BSC while cells settle can reduce edge effect, however, these conditions are suboptimal, even detrimental to cells. [3]**

## Objectives

**Test whether full-time optimal conditions for cell handling including a 37 degree working surface can reduce thermal differences and edge effect in 96-well plates.**

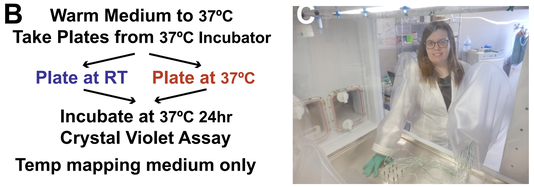
## References

1. Denmark J, Chessum B: Standardization of enzyme-linked immunosorbent assay (ELISA) and the detection of Toxoplasma antibody. Medical laboratory sciences 1978, 35(3):227-232.
2. Oliver DG, Sanders AH, Hogg RD, Hellman JW: Thermal gradients in microtitration plates. Effects on enzyme-linked immunoassay. Journal of immunological methods 1981, 42(2):195-201.
3. Lundholt BK, Scudder KM, Pagliaro L: A simple technique for reducing edge effect in cell-based assays. Journal of biomolecular screening 2003, 8(5):566-570

### Conflict of Interest Disclosure

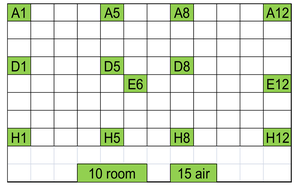
**The authors all are employees of BioSpherix, Ltd.**

## Experimental Design








**Experimental Design.** Studies were carried out in the Xvivo System (BioSpherix) using tanked, filtered, medical grade gases to control an atmosphere under different conditions. Figure 1A (L to R), entrance laminar flow hood, buffer chamber (air lock), double-sided processing chamber with controlled temperature floor and air, buffer chambers, a cell processing chamber with 2 black-doored incubators integrated, and a microscope chamber.
(B) Schematic of protocol. To look at the effect of plating conditions on edge effect, A549 human lung carcinoma cells were plated at either RT or 37ºC in processing chamber. Medium was pre-warmed to 37ºC and plates were moved to an incubator promptly after plating. Cells were incubated for 24 hr and assessed for cell density using a standard crystal violet assay. For plate temperature mapping, a temp mapper was placed in the processing chamber (B) Temp probes were placed in the indicated wells for simultaneous recordings immediately after plating plain medium (C) One probe was used to monitor room air and one was used to record the chamber air temp near the ceiling or the chamber floor. Temps were recorded for 20 min. Four trials were conducted with 4 96-well plates each trial.

## Results

**2A Room Temp Plating (Uncontrolled)**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.874 | 0.891 | 0.881 | 0.879 | 0.896 | 0.948 | 0.954 | 0.894 | 0.907 | 0.932 | 0.742 | 0.753 |
| B | 0.895 | 0.895 | 0.924 | 0.926 | 0.892 | 0.945 | 0.961 | 0.921 | 0.940 | 0.956 | 0.614 | 0.717 |
| C | 0.874 | 0.875 | 0.927 | 0.928 | 0.887 | Bl | Bl | 0.929 | 0.910 | 0.934 | 0.863 | 0.653 |
| D | 0.887 | 0.893 | 0.915 | 0.905 | 0.910 | Bl | Bl | 0.937 | 0.967 | 0.879 | 0.778 | 0.895 |
| E | 0.870 | 0.886 | 0.905 | 0.879 | 0.936 | 0.962 | 1.000 | 0.976 | 0.922 | 0.894 | 0.836 | 0.688 |
| F | 0.871 | 0.885 | 0.899 | 0.920 | 0.887 | 0.924 | 0.987 | 0.947 | 0.875 | 0.928 | 0.878 | 0.691 |
| G | 0.911 | 0.907 | 0.921 | 0.939 | 0.965 | 0.949 | 0.953 | 0.889 | 0.866 | 0.910 | 0.871 | 0.760 |
| H | 0.848 | 0.870 | 0.923 | 0.901 | 0.917 | 0.951 | 0.977 | 0.919 | 0.839 | 0.777 | 0.734 | 0.559 |

**B 37ºC Plating (Controlled)**

Cell Density: Low — Ave. — High

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.850 | 0.823 | 0.829 | 0.861 | 0.862 | 0.901 | 0.894 | 0.785 | 0.720 | 0.814 | 0.940 | 0.816 |
| B | 0.873 | 0.896 | 0.863 | 0.823 | 0.847 | 1.000 | 0.921 | 0.932 | 0.947 | 0.885 | 0.900 | 0.818 |
| C | 0.890 | 0.897 | 0.911 | 0.845 | 0.881 | Bl | Bl | 0.955 | 0.858 | 0.879 | 0.894 | 0.818 |
| D | 0.894 | 0.886 | 0.832 | 0.850 | 0.898 | Bl | Bl | 0.823 | 0.914 | 0.799 | 0.881 | 0.804 |
| E | 0.866 | 0.863 | 0.914 | 0.849 | 0.840 | 0.880 | 0.949 | 0.861 | 0.876 | 0.861 | 0.874 | 0.912 |
| F | 0.886 | 0.841 | 0.847 | 0.843 | 0.846 | 0.882 | 0.882 | 0.897 | 0.888 | 0.934 | 0.799 | 0.848 |
| G | 0.871 | 0.831 | 0.819 | 0.834 | 0.888 | 0.870 | 0.923 | 0.864 | 0.953 | 0.939 | 0.857 | 0.823 |
| H | 0.782 | 0.792 | 0.847 | 0.802 | 0.831 | 0.837 | 0.860 | 0.833 | 0.776 | 0.871 | 0.877 | 0.798 |

**Figure 2. Plating in a Controlled 37 Degree Chamber Reduced Edge Effect on Cell Density.** Heatmaps of cell density of A549 cells incubated for 24hrs after plating in either uncontrolled room temp or controlled 37ºC showed differences between plating in different temps, particularly in column 12. Plating was more even when performed at 37 rather than RT. % max values were averaged across both plates.

## Results (Cont.)

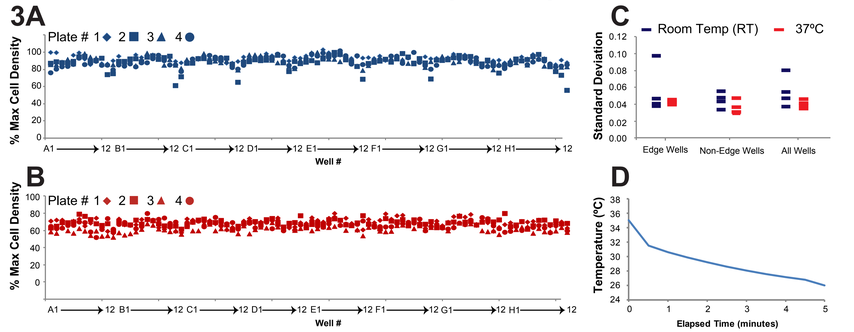



**Figure 3. Plating Cells in a Controlled 37 Degree Chamber Reduced Edge Effect - Four Plates.** Pooled data from all four plates in one trial showing clear edge effect in the pattern of cell density across the plates when plated in uncontrolled room air temperatures (A). When plated in controlled, constant temperatures (B), this edge effect pattern is reduced. (C) The standard deviation of the % max cell density of the edge wells, the non-edge wells, and all the wells of each plate was generally reduced by plating at 37ºC. (D) In a 50ml pipetting reservoir, medium rapidly cooled in the first five minutes at room temperature. This suggests that cells might chill during plating.

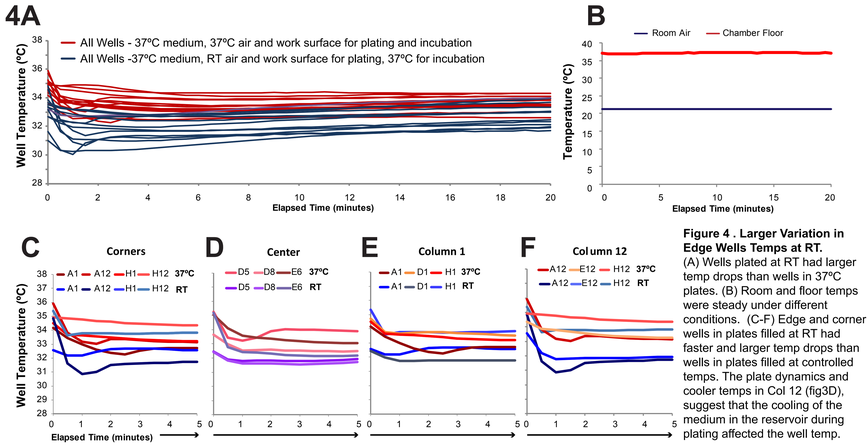



**Figure 4 . Larger Variation in Edge Wells Temps at RT.**
(A) Wells plated at RT had larger temp drops than wells in 37ºC plates. (B) Room and floor temps were steady under different conditions. (C-F) Edge and corner wells in plates filled at RT had faster and larger temp drops than wells in plates filled at controlled temps. The plate dynamics and cooler temps in Col 12 (fig3D), suggest that the cooling of the medium in the reservoir during plating affected the well temp.

## Conclusions

**Plating cells in 37ºC controlled conditions can reduce edge effect, allowing use of the whole plate, reducing the number of plates needed, and the amount of time needed to handle cells**