

Superior well-to-well Consistency with Eppendorf Cell Culture Plates

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Abstract

Consistency in the performance of cell-based assays is crucial in order to obtain reliable data. The edge effect is a common problem in 96-well formats leading to variances between read-outs of the outer wells compared to the center wells of a plate. Increased evaporation from the peripheral wells is assumed to be associated with this edge effect phenomenon.

The Eppendorf 96-Well Cell Culture Plate is equipped with an outer moat. It has been described before that filling the outer moat with liquid reduces evaporation to a minimum [1]. Here we show that the Eppendorf 96-Well Cell Culture Plate not only prevents evaporation but also minimizes well-to-well variability across the plate.

Introduction

In order to make valid scientific statements, the obtained data from cell-based assays must be reliable. Results of single wells can be influenced by their position within the plate. Cells growing in the edge wells often show a high variability in results due to evaporation and thermal inconsistency. This phenomenon is known as the edge effect. A very common method to circumvent the edge effect is to avoid seeding cells in the peripheral wells at the expense of sample throughput and efficiency (figure 1).

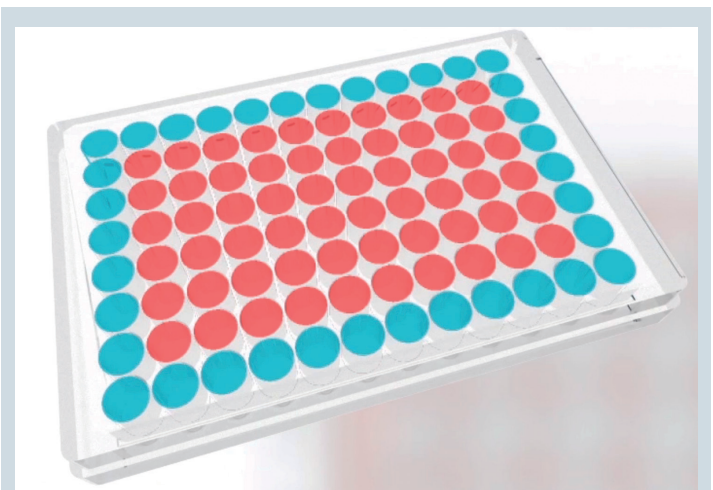
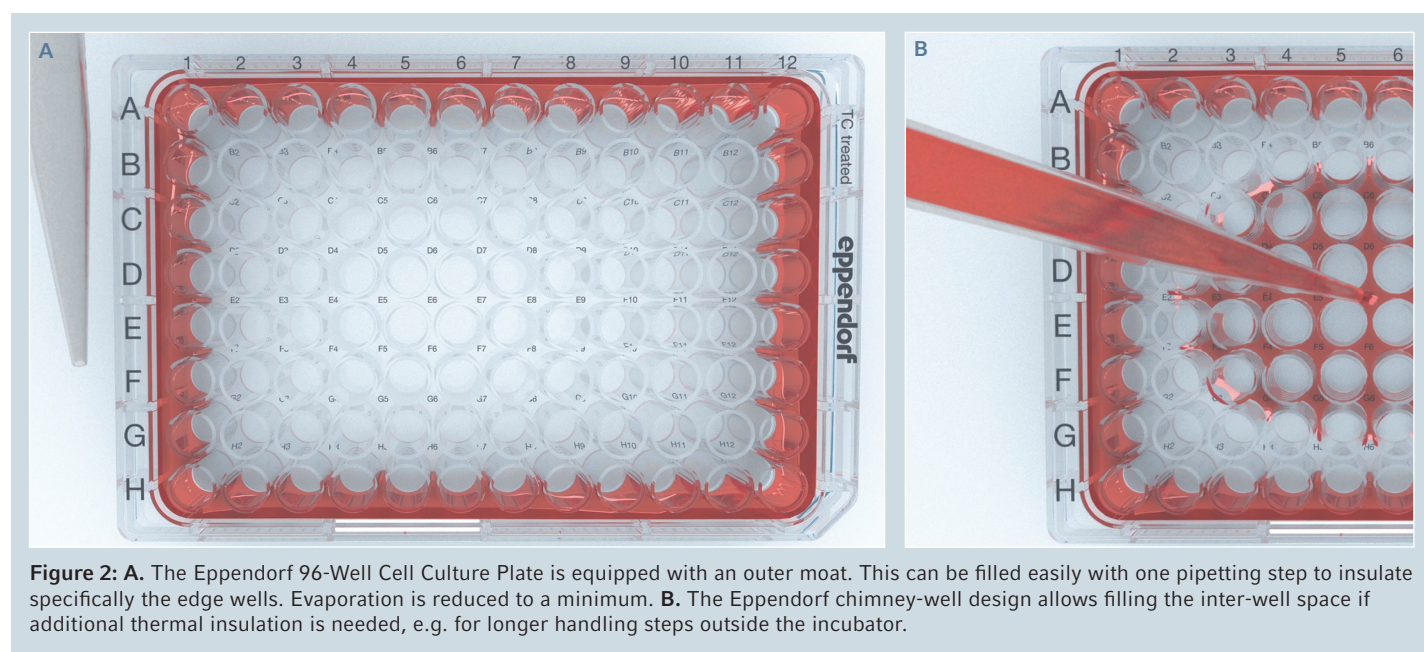


Figure 1: The edge effect affects the outer wells of 96-well formats (here highlighted in blue). By discarding the outer wells of a 96-well plate the number of wells for analysis is reduced to 60 which results in a decrease of 38% per plate. This means also a higher invest in consumables, incubator space, and time.

The Eppendorf 96-Well Cell Culture Plate has an outer moat which can be filled with liquid in just one pipetting step (figure 2A). It has been shown previously that evaporation can thus be reduced to a minimum [1]. The plate design also allows filling the inter-well space for additional thermal insulation, and as such becomes particularly relevant when the plate has to be handled for longer time periods outside the incubator environment (figure 2B).

Here we compare the performance of Eppendorf 96-Well Cell Culture Plates with plates from other manufacturers. We compare well-to-well variability in cell proliferation of two different cell lines using two standard cell proliferation assays.



Materials and Methods

For evaluating cell proliferation consistency in 96-well plates, the Eppendorf Cell Culture Plate as well as competitor plates were tested using two standard cell viability assays. Experimental results were verified by three independent replicates.

The outer moat of the Eppendorf plates was filled with 5 mL sterile water or a pre-warmed gelled solution (0.5% agarose). The plate from competitor C was prepared according to manufacturer's instructions. Prior to cell seeding the plates were equilibrated for 1 hour under standard conditions at 37 °C, 5% CO₂ in a humidified atmosphere in a Galaxy® CO₂ incubator.

Cells were thawed and pre-cultivated for at least 2 passages before use. For the first experiment HeLa cells (100 cells/well) were seeded in a final volume of 100 µL/well.

For the second experiment A549 cells (900 cells/well) were seeded in a final volume of 100 µL/well. To minimize pipetting variances caused by manual pipetting, semi-automatic cell seeding was performed using the epMotion® 96 (Dispensing mode, Asp 5, Disp 2). After seeding, plates were incubated at standard culture conditions as described above for seven days.

HeLa cell proliferation was analyzed after 7 days by WST-1 assay (Roche®) according to manufacturer's instructions. A549 cell proliferation was analyzed after 4 days by AlamarBlue® assay (Invitrogen®) according to manufacturer's instructions. All assay reagents were added by using the epMotion 96 (Pipetting mode, Asp 5, Disp 5).

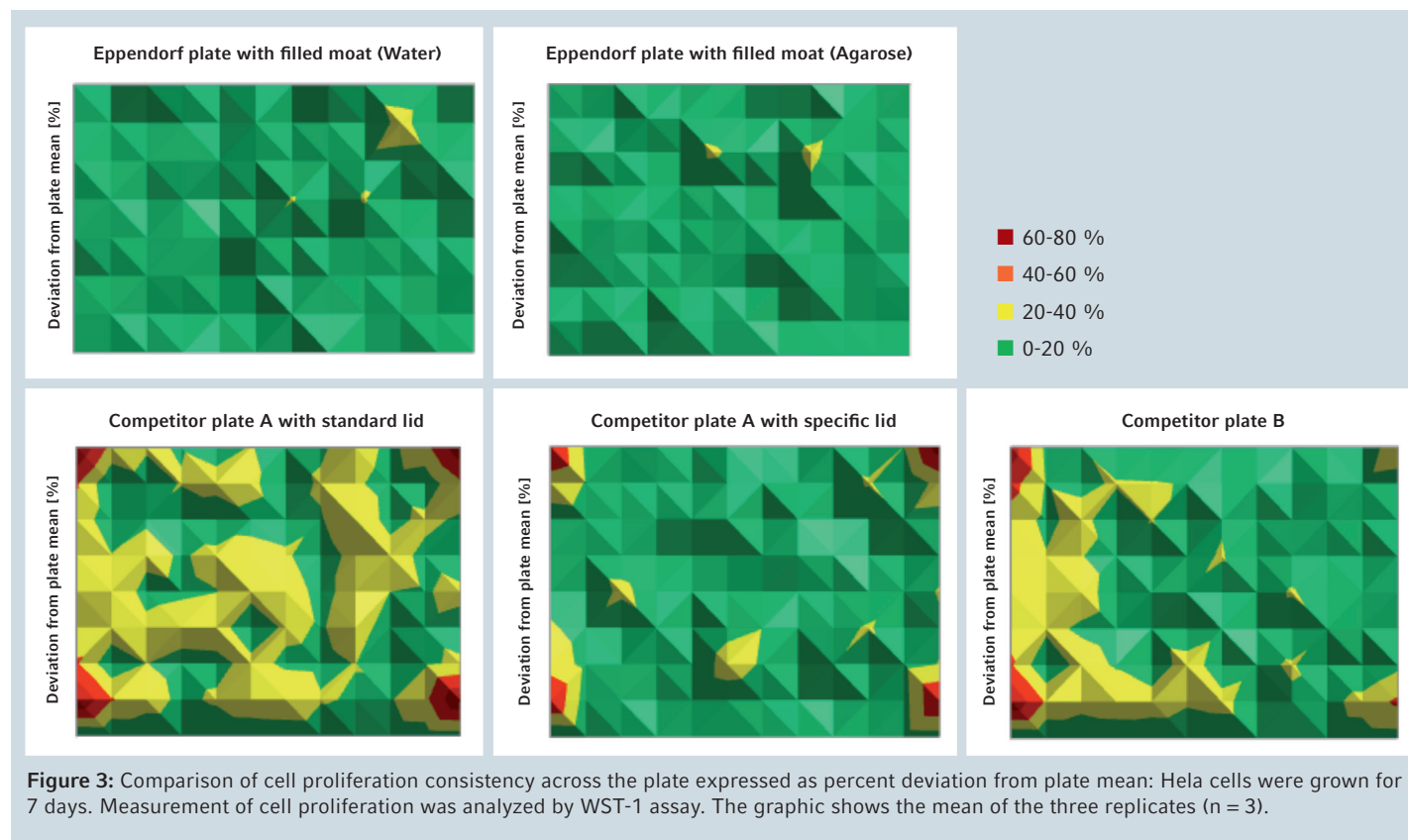
Results and Discussion

During longer incubation periods in 96-well formats, evaporation especially in the outer wells becomes apparent, because these wells are not completely surrounded by neighboring wells. It has been shown before, that filling the outer moat of the Eppendorf 96-Well Cell Culture Plate prevents this effectively [1]. Here we show that the insulation of edge wells also positively affects the well-to-well consistency.

In the first experiment we compared the Eppendorf Cell Culture Plate with standard cell culture plates. In the second experiment we compared the Eppendorf plate with a different plate that has four reservoirs that can be filled with liquid.

In both experiments equal starting cell numbers were used, and cells were grown for several days. Viable cell count was analyzed by either WST-1 or Alamar blue assay, two standard assays used for eukaryotic cells.

Figure 3 shows the result of the first experiment. The Eppendorf Cell Culture Plate with filled moat shows the most consistent cell growth across the plate compared to all other tested plates.

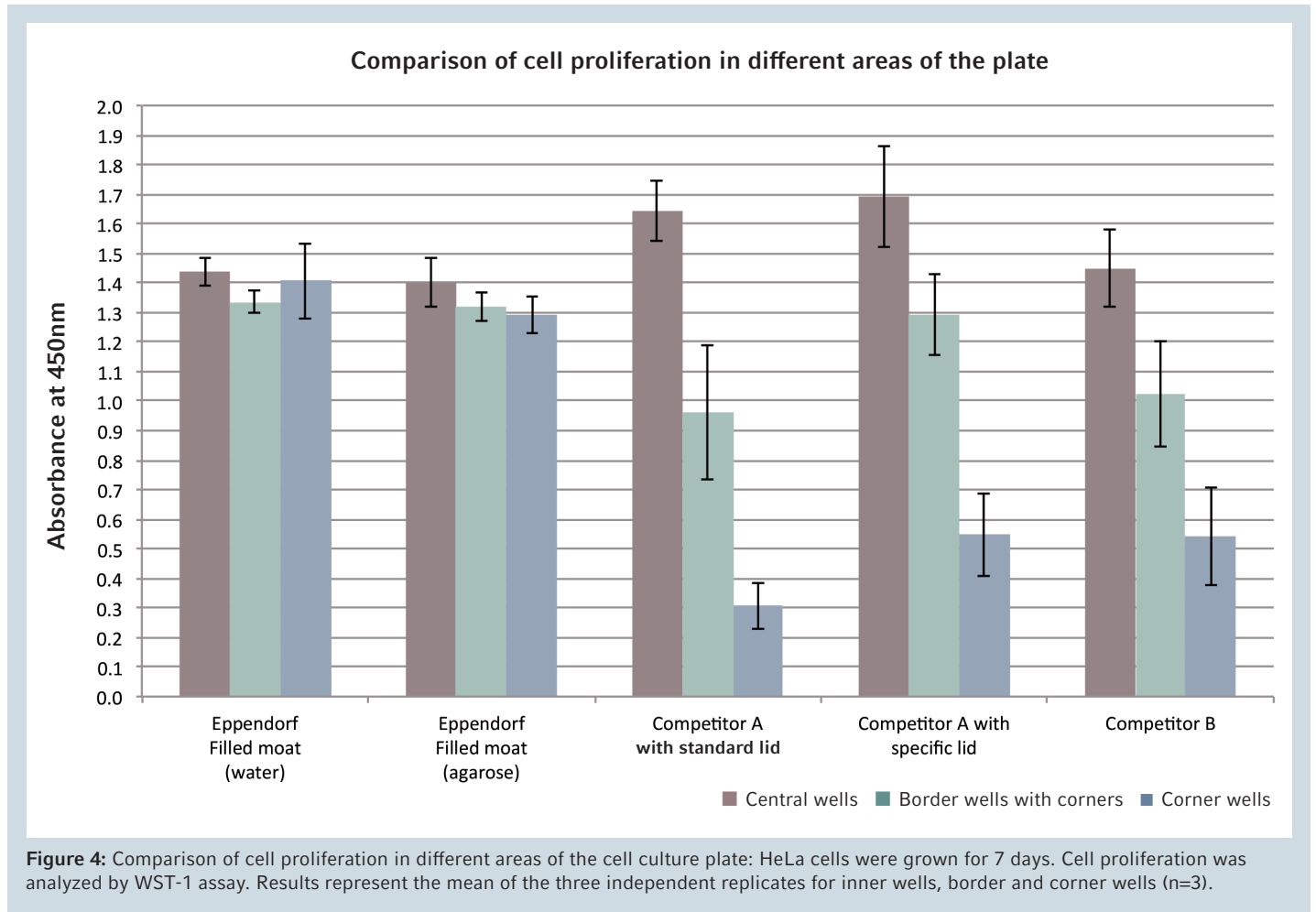


The edge effect does affect all tested competitor plates. Plates from supplier A and B show a strong edge effect. In the corner wells deviations of > 70% from the plate mean can be observed. Even in the center wells cell number variations of > 30% from plate mean are visible.

The competitor plate A with a specific evaporation reducing lid shows a more homogeneous cell growth across the plate, but the edge effect still is severe with a deviation from the plate mean of > 60%. Only the Eppendorf plate with the filled outer moat shows a homogeneous well-to-well consistency in cell proliferation across the whole plate including the edges.

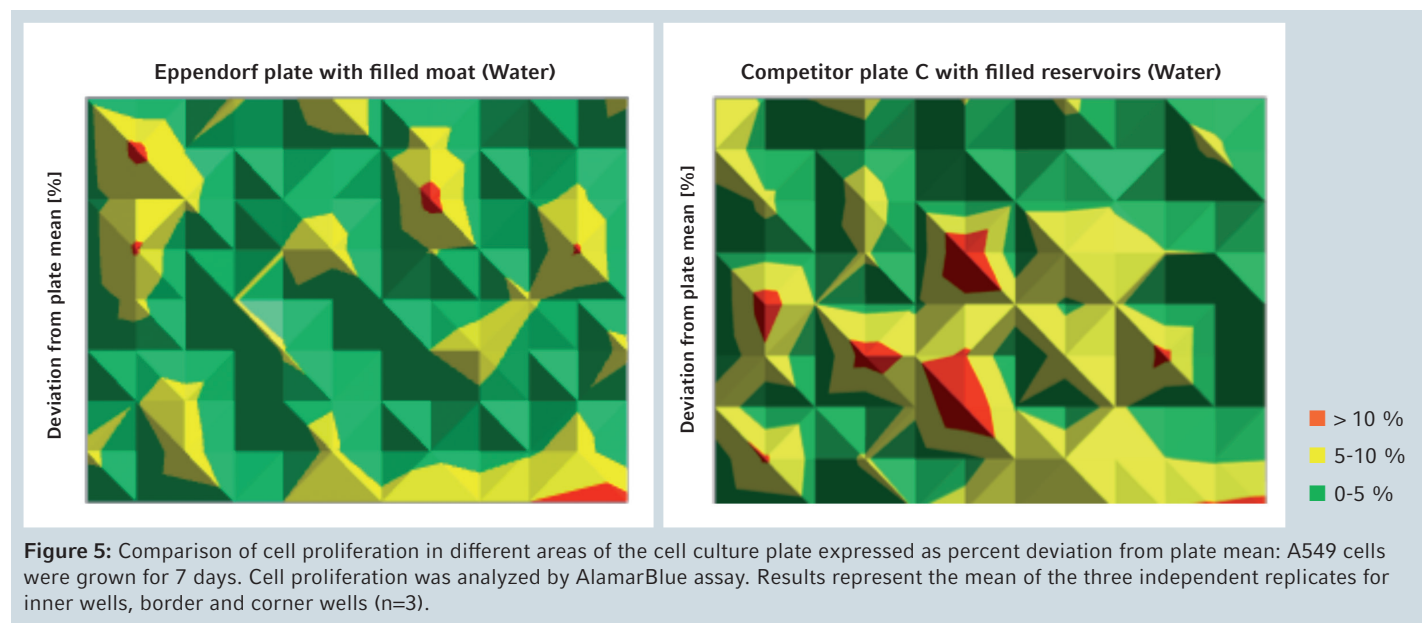
The moat of the Eppendorf Cell Culture Plate can be filled with either sterile liquid (e.g. water or buffer) or agarose solution (0.5%). Both options reduce the well-to-well variances effectively across the plate. A gelled solution can offer a handling advantage and the possibility to prepare the plates ahead of the experiments.

Figure 4 shows clearly that cell growth variability in all areas of the plate is minimized in the Eppendorf Cell Culture Plate whereas in the competitor plates, cell growth in the border and edge wells is impaired by the edge effect.



In the second experiment we compared the Eppendorf Cell Culture Plate with a plate that is equipped with four reservoirs that can be filled with liquid. Both plates prevent the edge effect and reduce growth deviations over the plate. As displayed in Figure 5 in the Eppendorf Cell Culture Plate

only 5% of the wells show deviations above 10% compared to 9% in competitor plate C. The maximum deviation in the Eppendorf plate is only 12%. The maximum deviation in the Competitor plate C is with 21% nearly twice as much as in the Eppendorf plate.



Conclusion

Filling the outer moat, either with a liquid or a gelled solution (like 0.5% agarose), leads to a superior well-to-well consistency in cell growth in the Eppendorf Cell Culture Plate. By this, the phenomenon of the edge effect can be minimized leading to reproducible performance in cell-based assays.

Literature

- [1] Wagener J, Plennevaux C: Eppendorf 96-Well Cell Culture Plate – A simple method of minimizing the edge effect in cellbased assays. Eppendorf Application Note 326; www.eppendorf.com

Ordering information

Description	Order no. international (230 V, 50/60 Hz)	Order no. North America (120 V, 50/60 Hz)
Eppendorf Cell Culture Plate , 96-Well, with lid, flat bottom, sterile, free of detectable pyrogens, RNase & DNase, DNA. Non-cytotoxic. TC treated, 80 plates, individually wrapped	0030 730.119	0030 730119
Galaxy® 170R CO₂ incubator (170 Liter, with high temperature disinfection)	C017311001*	C017211005
epMotion® 96 , semi-automated electronic pipette for parallel 96 channel microplate processing (without iPod® controller)	5069 000.012	5069000004

*Last digit is country dependent. For UK/HKG, change 1 to 2; for Australia, change 1 to 3; for China, change 1 to 4.

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