

# C.NEST™ | microbioreactor offers precisely controlled environments for mixing and static cell culture and significantly minimized evaporation and edge effects in well-plates

Chelsea Yuan-si Chou, BS, James Chao, MS, Robert Shih, MS, Charles Tsai, PhD  
CYTENA Bioprocess Solutions, Taipei City, Taiwan



## Abstract

Medium evaporation is a common problem when using microplates for high-throughput cell culture. Specifically, cell culture in wells experiencing significant evaporation often gives unreliable results in subsequent cell-based assays. A common problem regarding evaporation is called the edge effects, where peripheral wells tend to have higher evaporation compared to central wells. This phenomenon would cause inconsistent and unreliable results for experiments performed in multi-well plates, including for cell culture assays.

Here we proposed C.NEST as the solution to minimize the evaporation and inconsistency caused by the edge effects. In this study, we compared the levels of evaporation from 24-well plates placed in C.NEST against those in an incubator, under both static culture and mixing culture condition for 7 days.

Results show that in static culture, culturing in C.NEST results in lower evaporation compared to culturing in the incubator. Under mixing culture condition, use of C.NEST(with X.NEST Lid) not only results in lower liquid evaporation but a

more homogenous distribution of evaporation level between wells, reducing the variations caused by edge effects. In conclusion, C.NEST can minimize medium evaporation and edge effects by providing stable and controllable cell culture conditions for multi-well plate culture when compared to a regular cell incubator.

## Introduction

Cell-based assay is widely used in life science research such as for metabolism, aging, cancer, and neuroscience studies, as well as in pharmaceutical development processes such as drug screening, cell line development, tissue engineering or cell therapy. Furthermore, by utilizing multi-well plates, researchers can perform cell-based assay at high throughput scale with small volumes of valuable samples, which not only helps accelerate the development process but also lowers the cost of experiments. This makes high throughput cell-based assays in the multi-well plate a powerful tool for almost all biological applications.

However, a concern for using multi-well plate in long term cell culture is medium evaporation [1]. With medium evaporated and the volume decreased, the concentration of media components (nutrient or treated drug etc.) would increase, resulting in varying cell culture conditions between the wells and eventually leading to biased and unreliable assay results. Since plate peripherals (corners and edge) are more exposed to the surrounding, evaporation is often significantly higher for wells located in the peripheral region compared to wells in the central plate region. This well-documented phenomenon is known as the edge effects. In some studies, it has been shown that the uneven distribution of medium evaporation (edge effects) could cause problems in cell-based assays. For example, one study compared cell growth across different wells by comparing OD (optical density) measurements. When they plotted OD against row number and column number, they found that the relationship was a convex curve, indicating that there was systemic bias. [2] To circumvent the unreliable and/or biased assay results caused by edge effects, some researchers would sacrifice the wells located in the peripheral region and only seed cells in the central wells. However, this approach would result in reduced assay throughput, waste of incubator capacity and increased cost for consumables.

Various factors that affect evaporation have been discussed over the year, such as improving plate lid design, reducing humidity variability or lessening uneven thermal distribution of the cell culture environment. Here we propose using C.NEST microbioreactor to provide controllable and stable culture environment by minimize medium evaporation and edge effect. C.NEST microbioreactor have four independent and precisely controlled chambers for well-plate-based cell culture. Each C.NEST chamber is equipped with UV lights for sterilization, CO<sub>2</sub> and humidity sensors for environmental monitoring, and heating modules for temperature adjustment. Therefore, C.NEST can provide a more stable culture environment compared to regular incubators, which, due to frequent opening and closing, has unevenly distributed humidity and temperature profile within the incubator. In addition, C.NEST could perform not only static culture but also mixing culture by its patented reciprocating method (with X.NEST Lid), whereas a regular incubator requires an orbital shaker installed inside in order to perform mixing culture. For static culture, each C.NEST chamber can fit two 96/384-well or one 24-well plate. As for mixing culture, each C.NEST chamber can fit one 24 or 96-well plate with their matching X.NEST Lid. The specially designed X.NEST Lid not only equalizes the medium loss caused by mixing culture, but also enables evenly distributed mixing pressure, oxygen supply in each well of the multi-well plate.

In this article, we compared medium evaporation for 24 -well plates placed in C.NEST against plates placed in a regular incubator. We tested two culture conditions, static culture and mixing culture, in both C.NEST and a regular incubator. To study the level of edge effects in each group, we further classified the wells on 24-well plates into corner, edge and central subgroups for comparison. After 7 days of incubation, we evaluated the evaporation percentage (%) of each group and subgroup. Results show that C.NEST not only gives lower total evaporation in static and mixing cultures, but also generates more homogenous evaporation pattern between wells that minimizes the well-to-well variation caused by edge effects. These findings prove that C.NEST provides better cell culture and cell-based assay environment than conventional incubators by reducing concerns of evaporation or edge effects.

## Introduction

Culture Condition	Incubator	Culture Days
Static culture	Cell incubator	7
	C.NEST microbioreactor 7	7
Mixing Culture	Cell incubator (orbital plate shaker) 7	7
	C.NEST microbioreactor (with X.NEST Lid) 7	7

### A. Methods of cultivation

To evaluate evaporation of media in static culture and mixing culture in different incubators, we placed 4 groups of 24-well cell culture plates (GREINER, CELLSTAR, No. 662102) (n=3) into C.NEST and a standard cell incubator (ASTECH, SCA-165DS) according to the chart above. To provide mixing culture, an orbital plate shaker (UENAGA, COSH6, No. 3-6560-01) was placed in the forementioned incubator with 130 rpm rotation speed. As for C.NEST, 24-well plates were used with X.NEST Lid and the reciprocate mixing speed was set to 10 seconds/cycle (continuous mode). All plates were incubated under standard cell culture conditions (37°C, 5% CO<sub>2</sub>, humidified atmosphere) in either the standard cell incubator or the C.NEST chamber.

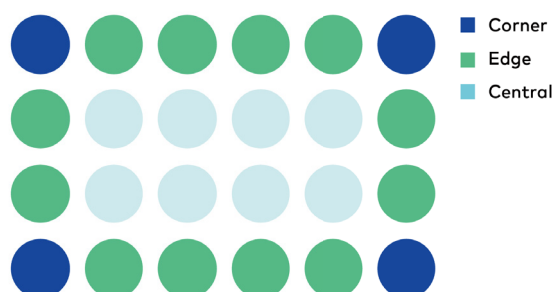
### B. Measurement of evaporation

At the beginning of the experiment, each plate was filled with 1400 µL of PBS (phosphate buffered saline) per well. After days of incubation, plates were removed from the cell incubator and the C.NEST chamber. The plates were allowed to equilibrate to room temperature for 40 minutes before measurements.

To evaluate the evaporation percentage of each well, we removed PBS inside the individual wells one at a time and weighed the plates using an electronic balance (SHIMADZU, ATX224). From the weight change measured by the balance, we can acquire the value of "g total PBS remained in well". Then, with the formula listed below, we calculate the evaporation % in each well:

$$Evaporation \% = \left(1 - \frac{g \text{ total PBS remained in well}}{g \text{ total PBS added in well}}\right) \times 100$$

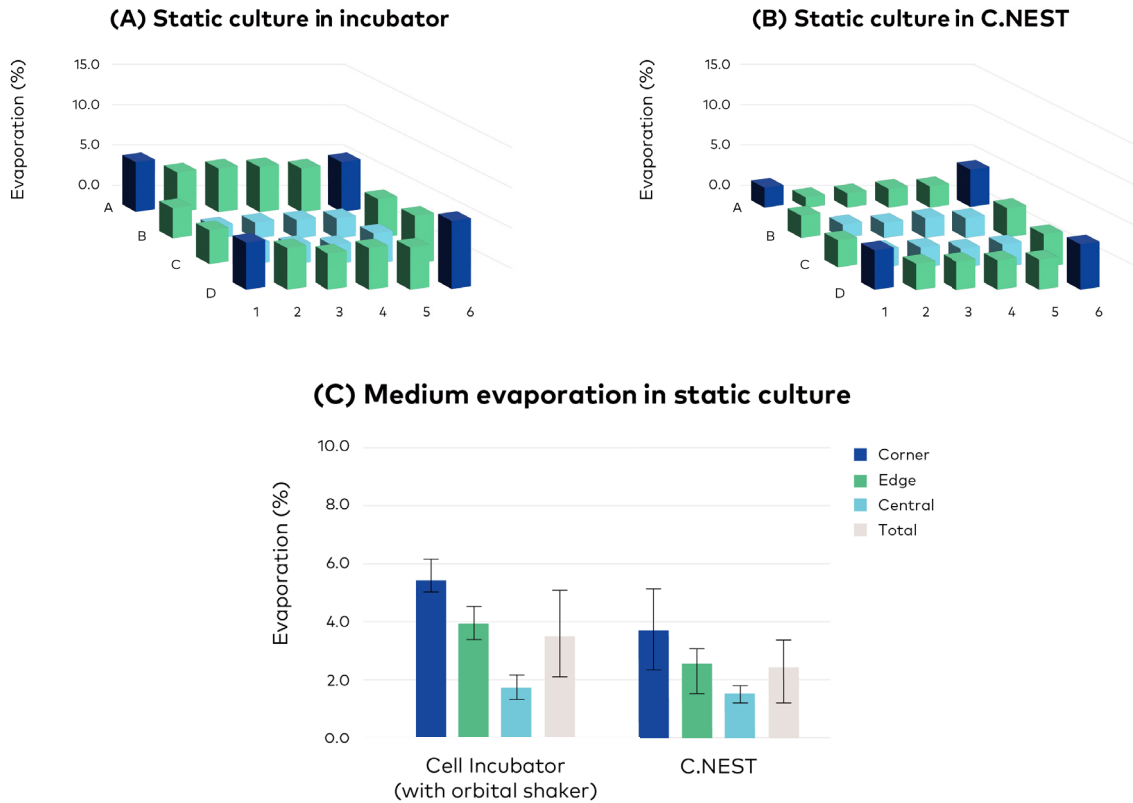
To compare the level of edge effects in each group, we further divided the wells on multi-well plates into three subgroups: corner, edge, and central region wells. The mean evaporation levels for the subgroups were calculated and depicted in the following figures.



## Results and discussion

After 7 days of incubation, we analyzed the PBS evaporation of individual wells in the plates. In addition, we averaged the evaporation percentage of central, edge and corner wells to observe the degree of edge effects in each group. Average total evaporation was also calculated to evaluate liquid lost in each group.

In 24-well plate group, the evaporation percentage were generally lower than 5% in static culture group (**Figure 1**). However, it is apparent that 24-well plates placed in C.NEST had lower total evaporation and less evaporation level difference between corner, edge and central wells compared to the incubator group (**Figure 1C**).



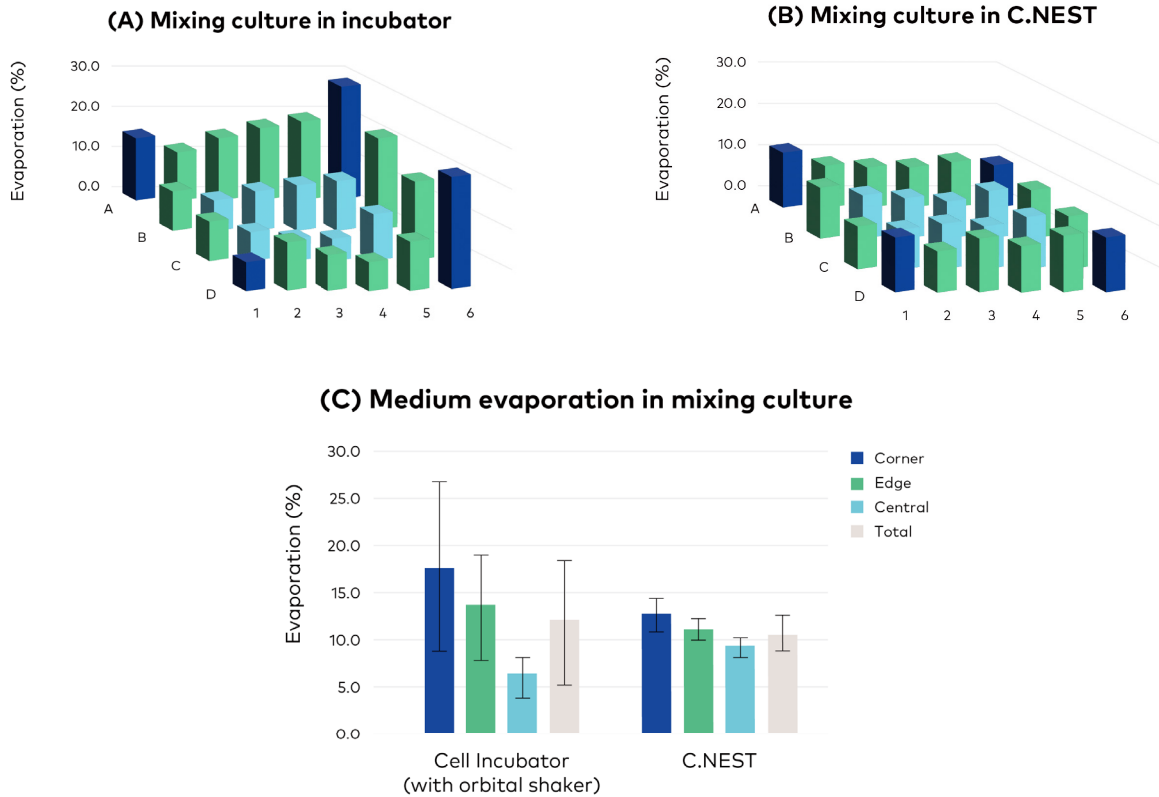
**Figure 1. Comparison of evaporation % in 24-well plates under static culture conditions.**

Individual well evaporation % for plates(n=3) incubated in(A) a standard incubator or (B) C.NEST for 7 days. (C) Mean evaporation % of corner wells(n=12), edge wells(n=36), central wells(n=24) and all wells

TECHNICAL NOTE

As for the mixing cell culture condition, evaporation was higher overall compared to that of the static culture. For plates placed in the incubator (**Figure 2A**), serious edge effects were shown as some corner and edge wells had much higher evaporation levels compared to the central wells. Whereas for plates placed in C.NEST (**Figure 2B**), the edge

effects were effectively minimized due to the homogenous distribution of evaporation between corner, edge and central wells. The overall average evaporation level (**Figure 2C**) was also lower in C.NEST.



**Figure 2. Comparison of evaporation % in 24-well plates under mixing culture conditions.** Individual well evaporation % for plates(n=3) **(A)** placed on an orbital plate shaker in a standard incubator or **(B)** under reciprocate mixing with 10 sec/cycle in a C.NEST chamber(with X.NEST Lid) for 7 days of incubation. **(C)** Mean evaporation % of corner wells(n=12), edge wells(n=36), central wells(n=24) and all wells.

## Conclusion

As demonstrated in this article, C.NEST shown to provide a more stable culture environment compared to a regular incubator in static culture. For mixing culture, C.NEST with X.NEST Lid minimized evaporation and provided a more homogenous evaporation distribution, which helps researchers avoid any negative influences due to edge effects such as inconsistent media concentration, variation in temperature or oxygen level between wells in cell-based assays or cell culture. In conclusion, we believe C.NEST is the most reliable solution for your high throughput cell culture and cell-based assays.

## References

1. Walzl, Angelika et al. "A Simple and Cost-Efficient Method to Avoid Unequal Evaporation in Cellular Screening Assays, Which Restores Cellular Metabolic Activity." (2014). [DOI: 10.30845/ijast](https://doi.org/10.30845/ijast)
2. Faessel, H M et al. "Parabolic growth patterns in 96-well plate cell growth experiments." In vitro cellular & developmental biology. Animal vol. 35,5 (1999). [DOI: 10.1007/s11626-999-0071-z](https://doi.org/10.1007/s11626-999-0071-z)



©2023 BICO AB. All rights reserved. Duplication and/or reproduction of all or any portion of this document without the express written consent of BICO is strictly forbidden. Nothing contained herein shall constitute any warranty, express or implied, as to the performance of any products described herein. Any and all warranties applicable to any products are set forth in the applicable terms and conditions of sale accompanying the purchase of such product. BICO provides no warranty and hereby disclaims any and all warranties as to the use of any third-party products or protocols described herein. The use of products described herein is subject to certain restrictions as set forth in the applicable terms and conditions of sale accompanying the purchase of such product. BICO may refer to the products or services offered by other companies by their brand name or company name solely for clarity and does not claim any rights to those third-party marks or names. BICO products may be covered by one or more patents. The use of products described herein is subject to BICO's terms and conditions of sale and such other terms that have been agreed to in writing between BICO and user. All products and services described herein are intended FOR RESEARCH USE ONLY and NOT FOR USE IN DIAGNOSTIC PROCEDURES.

The use of BICO products in practicing the methods set forth herein has not been validated by BICO, and such nonvalidated use is NOT COVERED BY BICO'S STANDARD WARRANTY, AND BICO HEREBY DISCLAIMS ANY AND ALL WARRANTIES FOR SUCH USE. Nothing in this document should be construed as altering, waiving or amending in any manner BICO's terms and conditions of sale for the instruments, consumables or software mentioned, including without limitation such terms and conditions relating to certain use restrictions, limited license, warranty and limitation of liability, and nothing in this document shall be deemed to be Documentation, as that term is set forth in such terms and conditions of sale. Nothing in this document shall be construed as any representation by BICO that it currently or will at any time in the future offer or in any way support any application set forth herein.

Edited version: September 2023 | CBS\_PUB\_CNEST\_Tech-Note-02\_Digital