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EZHold | A Standardized Machine to Lower Liquid Loss after Culturing in C.BIRD and S.NEST Microbioreactor Systems

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Abstract

To meet the growing demand for the development of biomedicines and biotechnologies, a system that is highly effective and efficient is needed. Cell-based assays, especially for drug discovery, require precise measurements of fluid volume. To obtain a reliable and meaningful result, procedures must be standardized. The C.BIRD™ and S.NEST™ systems are high-throughput microbioreactors that standardize and automate early-stage cell screening. Each system is associated with its own functional lid and specific tubes for different types of microplates. However, we have found that manual opening of the lid by different testers results in different amounts and sizes of residual droplets, which contributes to unreliable data. In this application note, we demonstrate how the EZHold™ effectively minimizes droplets and prevents liquid from remaining on functional lids.

Introduction

In vivo studies are becoming increasingly important in the field of biology because they mimic the real conditions of an animal's body, giving researchers a more accurate understanding of the complexity of organisms. On this basis, our C.BIRD and S.NEST devices provide 3D cell culture environments that promote cell proliferation and allow real-time monitoring of each well. These products can be used with both 24-well and 96-well formats and are associated with proprietary functional lids that are distinct from the tubes.

In this application note, we found that the experimental data were not always consistent from tester to tester. Manual opening of the lids could be a reason for the high liquid loss from plates, as many water droplets sometimes remain on the lid. To maximize the efficiency of high-throughput screening, a tool that reduces the amounts of residual droplets is needed. The EZHold, an instrument that mainly consists of a knob and a platform to place the microtiters plate, was developed to resolve this issue by separating the lid from the microplate in a standardized and consistent manner. The EZHold not only reduces the amount of residual water but also minimizes variations between different users. Our experiment demonstrated that using the EZHold helps reduce residual droplets on the lid and maintains consistent and comparable liquid volume in the wells.

Materials and methods

A. Samples and mode of mixing

The test required three testers with experience conducting biological experiments to separate a 24-well lid and a 96-well lid from their respective plates manually and with the EZHold. Five sets of data (n=5) were collected for each tester. All data were measured by the same person to avoid measurement errors. After adding 1,400 µL distilled water to each well of a 24-well plate, and 200 µL distilled water to each well of a 96-well plate, we used the C.BIRD system to drive the mixing for 5 minutes, mimicking both the C.BIRD and S.NEST operation mode at a mixing rate of 25 seconds for the 24-well plate and 15 seconds for the 96-well plate, respectively.

B. Measurement of residual liquid

All measurements were taken by the same person for data consistency. As part of the experiment, the weight of the 24-well plate (GREINER, CELLSTAR, No. 662102) was recorded before and after it was filled with 1,400 µL distilled water. Similarly, the weight of the 96-well plate (CORNING 96-well Clear Flat Bottom Microplate, No.3599) was recorded before and after it was filled with 200 µL distilled water. The lid was separated by three testers in their individual way after 5 minutes of mixing controlled by the C.BIRD. The plates were then weighed again after the experiment to determine the volume of residual liquid on the lid.

Results and discussion

Compared to using the EZHold, we found that different testers often left a large deviation and a large amount of water on the lid when they manually opened the lid. To demonstrate the difference between using the EZHold and opening the lid manually, we asked three testers with biological backgrounds to perform the experiments (**Figure 1** and **Figure 2**). The results showed that regardless of the testers, manually opening the lid in both the 24-well plate and 96-well plate resulted in higher fluid loss and a greater chance of errors. Furthermore, data also differed significantly between the testers.

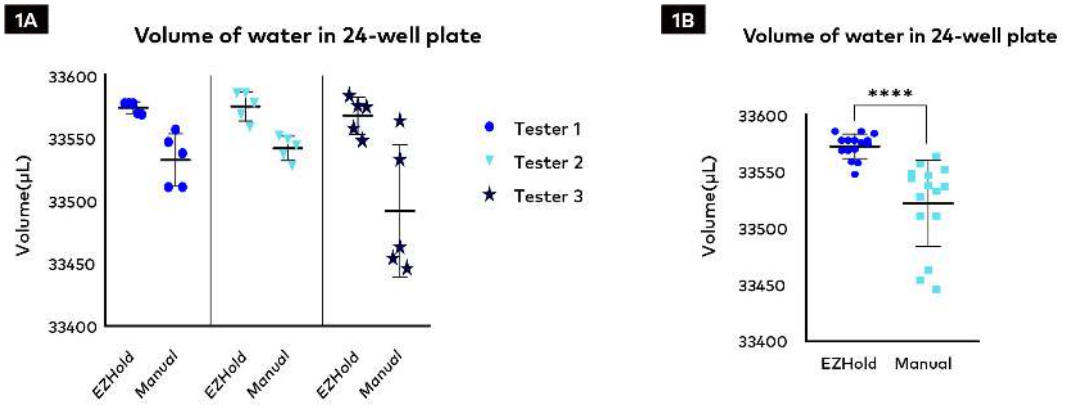

Figure 1. *(A) The volume of water left in the 24-well plate. (B) Comparison between the EZHold and manual opening of 24-well lid. Unpaired t-test and ordinary one-way ANOVA, ****p < 0.0001.*

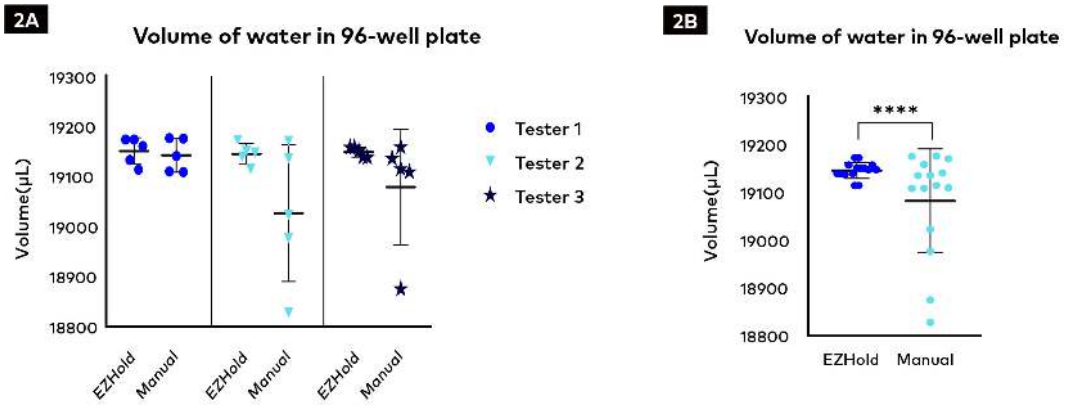

Figure 2. *(A) The volume of water left in the 96-well plate. (B) Comparison between the EZHold and manual opening of 96-well lid. Unpaired t-test and ordinary one-way ANOVA, ****p < 0.0001.*

We found similar discrepancies in liquid volume between manual separation and the EZHold in both 24-well and 96-well experiments. The data from the EZHold, regardless of the testers, illustrated an even and consistent trend. On the other hand, the manual opening of the lid showed a larger variation possibly due to the different speeds, degrees and ways each tester opened the lid.

Fluid loss from microtiter wells contributes to experimental error and compromised data, especially in drug discovery experiments, cultivation of drug-resistant strains, and others. Based on this empirical study, we found that manual removal caused uneven water droplets to remain on the lids, which in turn led to uneven amounts of liquid in each well. In order to lower the experimental error and obtain reliable data, we suggest scientists use the EZHold when they need to add supplements or other components to wells after performing cell cultures in the C.BIRD and S.NEST systems.

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