APPLICATION NOTE

# Cellometer X2 fluorescent cell viability counter: A rapid method of *Candida albicans* culture sample stability determination.


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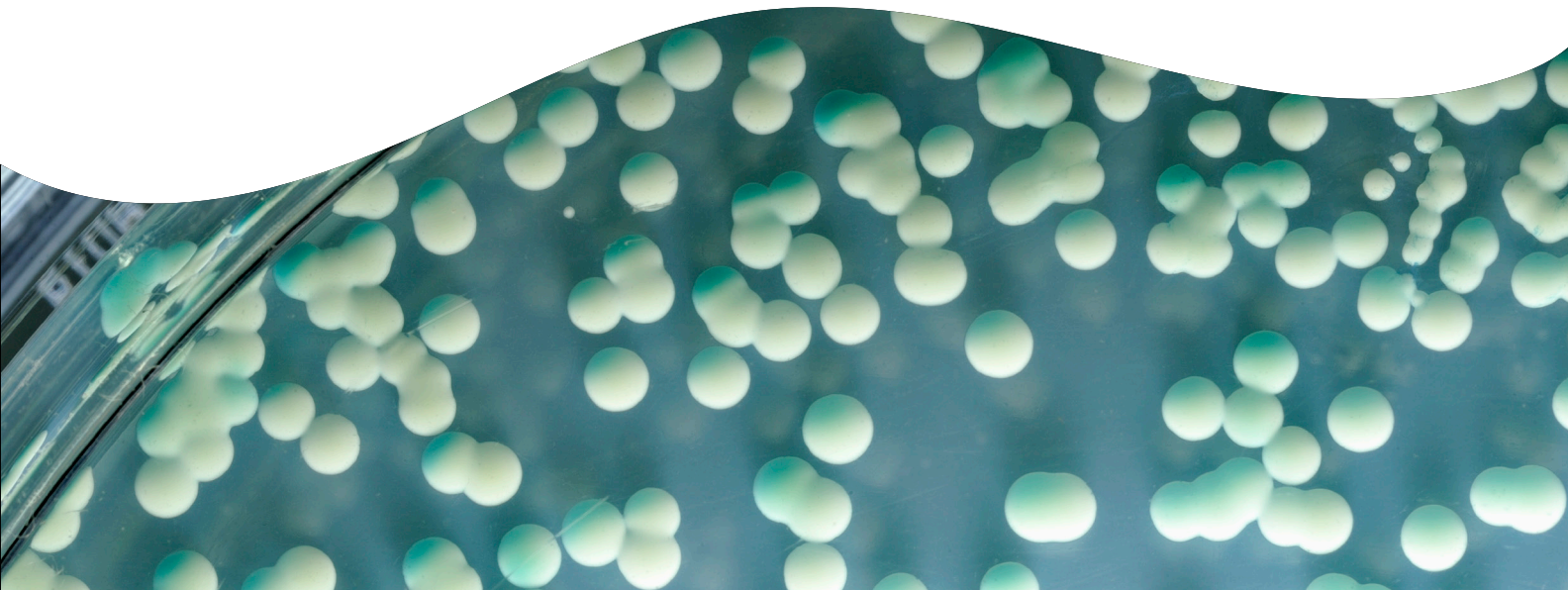
## Introduction

*Candida albicans (C. albicans)*, a common cause of candidiasis, ranks high among the WHO's critical priority pathogens in 2022 [1]. This invasive fungal infection poses severe health threats if not treated properly. It is also frequently used in studies related to infectious diseases, antifungal resistance, and host-pathogen interactions [2]. Ensuring accurate concentration and viability of *C. albicans* cultures is crucial in research and clinical contexts, as the stability of these cultures directly influences the reliability and reproducibility of experimental outcomes.

Different storage methods can have various effects on the viability of fungal cultures over time. Commonly used methods for preserving *C. albicans* include maintaining cultures on agar plates, storing resuspended cultures in phosphate-buffered saline (PBS) at low temperatures, or storing frozen cultures in glycerol. Previous studies have shown that *C. albicans* cultures can maintain high viability on agar plates, and in PBS for up to 4 weeks when stored at 4°C, while cryopreservation in liquid nitrogen or ultra-low freezers is highly recommended for long-term storage [3]. The standard method of determining the concentration and viability of *C. albicans* cultures is manual counting using a hemacytometer under a microscope. It is cost-effective and allows for direct visualization of cells; however, it is also labor-intensive and prone to user error, which limits its ability to provide consistent results in a timely manner. In modern cell culture laboratories, a rapid and reliable test for measuring cell viability is highly desirable.



Cellometer™ X2 fluorescent cell viability counter (herein referred to as Cellometer X2) is an imaging cytometry system that automatically measures cell concentration and viability using brightfield (BR) and dual fluorescence (FL) labeling methods. A prior study has demonstrated that the instrument provides reliable concentration and viability measurements for yeast in corn mash directly from operating fermenters [4]. Cellometer X2 has a concentration range from 5 x $10^5$ to 5 x $10^7$ cells/mL, depending on the size of the cell [5]. Here, we performed a dynamic range study and sample stability studies using the Cellometer X2 instrument with *C. albicans* cultures stored under three common methods. We aim to compare the ability of these storage methods to maintain high viability of *C. albicans* cultures, and to present a rapid solution for laboratories that routinely require viable cultures for their experimental workflows.

## Materials and methods

### *C. albicans* fresh culture on agar plate preparation

*C. albicans* frozen stock received from BEI (Cat. #NR-29445) was seeded on Candida BCG agar plate (Edge Biologicals) for one day at 27°C. After the formation of visible colonies, they were collected and resuspended in 1X PBS buffer for initial measurement. The plate was stored at 4°C over the course of 31 days, with Day 0 designated as one day after the plate was seeded. Individual samples collected on various days (Day 0, 8, 16, 23, and 31) were resuspended in PBS prior to measurement by the Cellometer X2 instrument (Revvity, Cat. # CMT-X2-S150).

### *C. albicans* fresh culture in PBS preparation

Fresh *C. albicans* colonies were grown on an agar plate as described above, followed by resuspension in 1X PBS buffer for initial measurement. The resuspended fresh culture stock was stored at 4°C over the course of 29 days and measured by the Cellometer X2 on Day 0, 7, 14, 21, and 29.

For the dynamic range study, *C. albicans* culture in PBS was diluted with the yeast dilution buffer (Revvity, Cat. #CSK-0102-2mL) to the expected concentrations at 1.62 x $10^8$ and 1 x $10^8$ cells/mL, followed by 5-fold serial dilution till it reached the lowest at 1.6 x $10^5$ cells/mL.

### *C. albicans* frozen culture preparation

Fresh *C. albicans* colonies were grown on an agar plate as described above, followed by resuspension in 1X PBS. 20% of sterile glycerol was added to the resuspended stock at a 1:1 ratio. The glycerol stock was stored at -80°C and underwent a total of 8 freeze/thaw (F/T) cycles. The freshly made glycerol culture without F/T was designated as F/T 0. Individual samples collected from each freeze/thaw cycle were measured by the Cellometer X2.

### Cell staining and quantification using Cellometer X2

At each testing time point or testing concentration of all studies, *C. albicans* culture was diluted with the yeast dilution buffer at a 1:4 ratio. 20 µL of each diluted sample was mixed with 20 µL of the yeast acridine orange/propidium iodide (AO/PI) dye (Revvity, Cat. # CSK-0102-2mL) and subsequently inserted into the Cellometer X2 instrument. The cells were allowed to settle inside the chamber for 3 minutes of incubation prior to image acquisition and analysis. Cellometer X2 utilized brightfield and dual-fluorescent imaging modes to automatically generate stock concentration, viability, and fluorescent intensity of target cells [6].

## Results

### The dynamic range of Cellometer X2 for *C. albicans*

*C. albicans* culture with concentration series ranging from 1.76 x $10^5$ to 1.58 x $10^8$ cells/mL were measured, and the results were plotted (Figure 1). The measured concentrations and dilution factors between 2.61 x $10^5$ to 1.04 x $10^8$ cells/mL showed a linear relationship with an $R^2$ of 0.9991. Therefore, the dynamic range for counting *C. albicans* cells on the Cellometer X2 is between 2.61 x $10^5$ to 1.04 x $10^8$ cells/mL. Although this upper limit is higher than the instrument specifications range limit of 5 x $10^7$ cells/mL [5], the results are still in the linear relationship, providing reliable measurements. Similar concentrations were used in stability studies as described below.

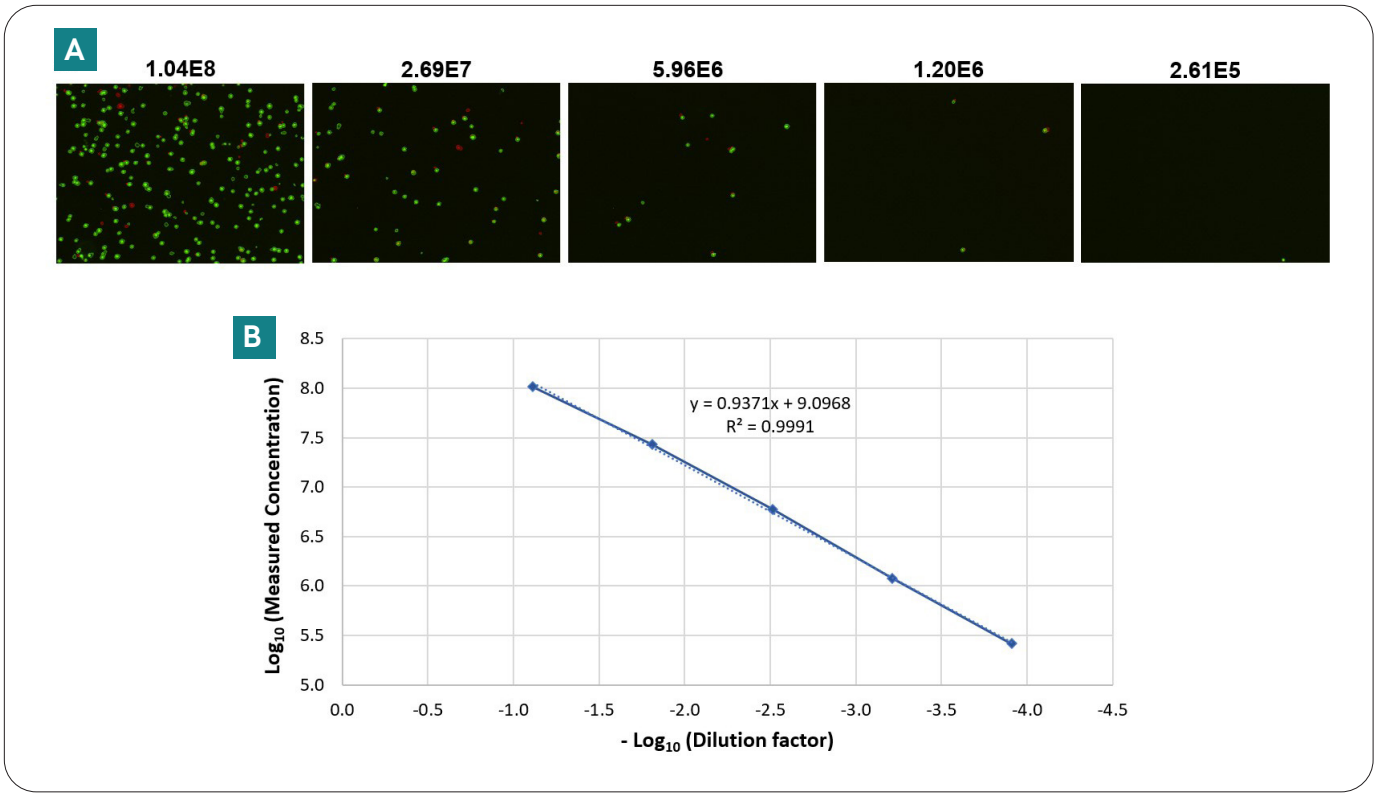



Figure 1: The dynamic range of Cellometer X2 for *C. albicans*. (A) Fluorescent images of *C. albicans* culture. Green: live cell marker. Red: dead cell marker. Scale bar not shown. (B) Cellometer X2 Dynamic range for counting *C. albicans*.

### Stability of *C. albicans* fresh culture in 1X PBS stored at 4°C

Based on live and dead cell counts, the viability of the fresh culture dropped slightly from 99.0% to 91.0% over the test period of 29 days (Figure 2). It suggested that *C. albicans* fresh culture in 1X PBS is highly viable (>80%) after 29 days of storage at 4°C, consistent with findings from previous studies [3].

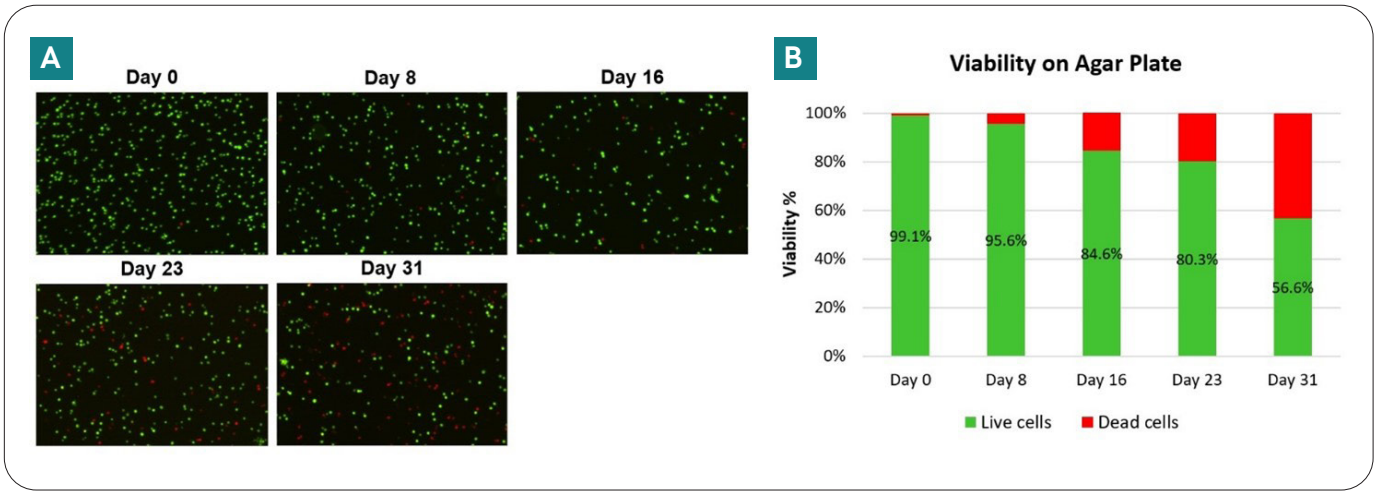



Figure 2: Stability of *C. albicans* fresh culture in 1X PBS stored at 4°C from Day 0 to Day 29. (A) Fluorescent images of *C. albicans* fresh culture. Green: live cell marker. Red: dead cell marker. Scale bar not shown. (B) Viability of *C. albicans* fresh culture.

### Stability of *C. albicans* culture on agar plate stored at 4°C

The viability of the culture stayed above 80% from Day 0 to Day 23 but reduced to 56.5% on Day 31 (Figure 3). It suggested that *C. albicans* culture on agar plate lost viability gradually over 31 days at 4°C, as evidenced by the abundance of dead cells on Day 31 (Figure 3A).

Compared to fresh culture in 1X PBS, culture on agar plate showed a more pronounced decrease in viability. A richer plate medium might be needed to sustain *C. albicans* culture for a longer period.

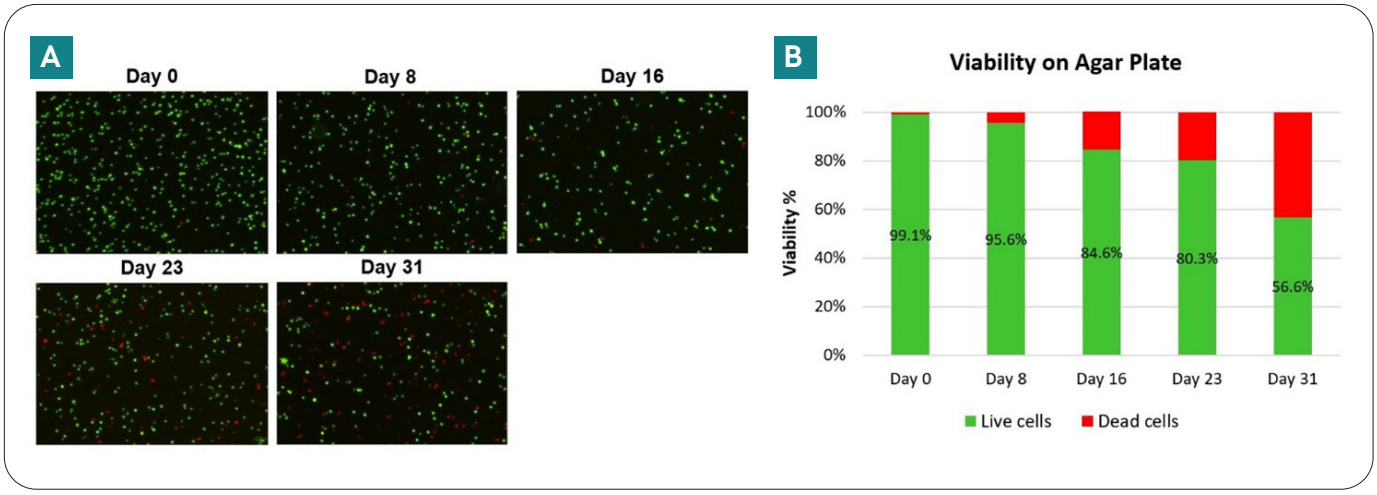



Figure 3: Stability of *C. albicans* culture on agar plate stored at 4°C from Day 0 to Day 31. (A) Fluorescent images of *C. albicans* culture. Green: live cell marker. Red: dead cell marker. Scale bar not shown. (B) Viability of *C. albicans* culture.

### Stability of *C. albicans* glycerol culture stock over freeze/thaw cycles

The viability of the frozen glycerol culture remained stable at around 80% throughout 8 F/T cycles (Figure 4). It showed that repeat F/T does not impact *C. albicans* frozen culture in 10% glycerol viability for up to 8 cycles.

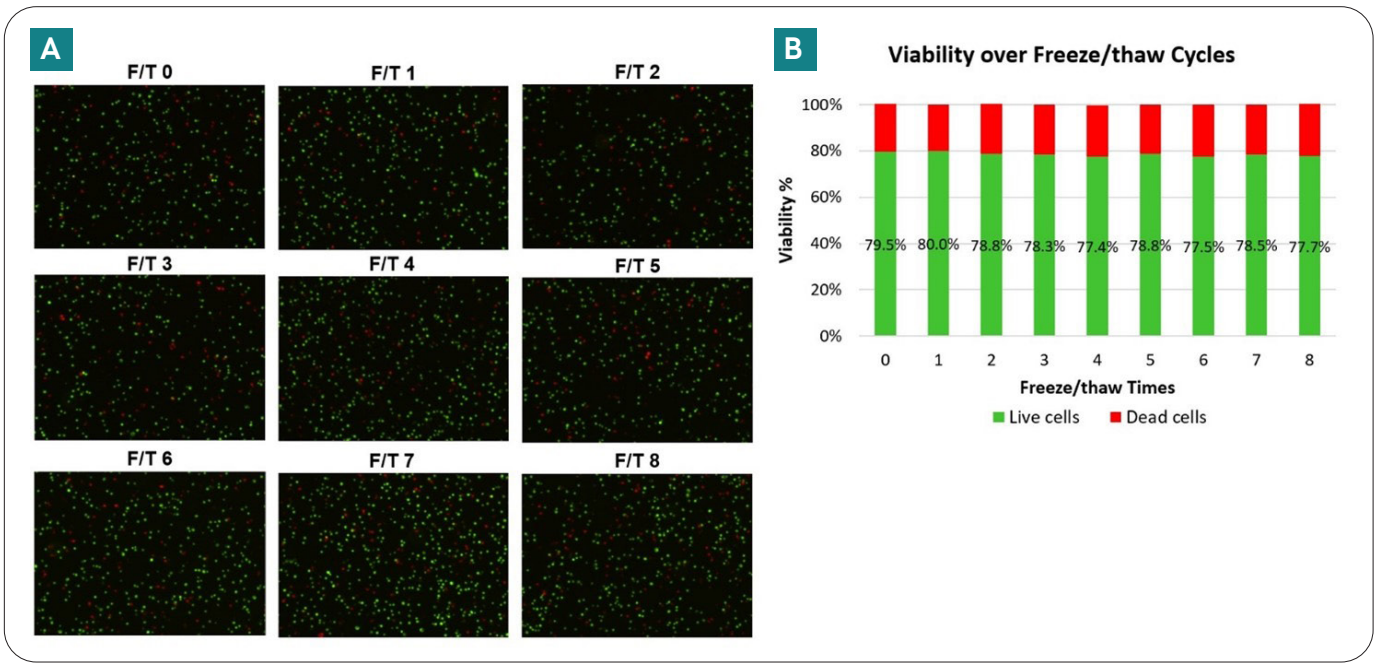



Figure 4: Stability of *C. albicans* frozen culture stock in 10% glycerol over 8 freeze/thaw cycles. (A) Fluorescent images of *C. albicans* culture. Green: live cell marker. Red: dead cell marker. Scale bar not shown. (B) Viability of *C. albicans* culture.

## Conclusion

In this study, the stability of *Candida albicans* cultures under various storage conditions was evaluated using Cellometer X2 fluorescent cell viability counter.
The Cellometer X2 dynamic range for counting *C. albicans* on the Cellometer X2 is 2.61 x $10^5$ to 1.04 x $10^8$ cells/mL. The findings indicated that *C. albicans* culture in 1X PBS, and on agar maintains high viability for at least 3 weeks, and the frozen culture in 10% glycerol is highly viable through multiple freeze/thaw cycles. Furthermore, the study demonstrates that Cellometer X2 can be used as a rapid and reliable method for quantification and sample stability testing of fungal cultures. The Cellometer X2 can provide immediate insights into fungal culture sample quality and quantity prior to analytical studies.

## References

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