application note

Using the LUNA™ Reusable Slide for Accurate Cell Counting with Automated Cell Counters

INTRODUCTION

Cell counting is an essential step in routine cell maintenance and for obtaining accurate and consistent experimental results. In recent years, automated cell counters have become more popular because affordable instruments have become increasingly available at competitive prices. However, many researchers still use the hemocytometer to avoid the expenses associated with the use of disposable slides. To overcome this problem, Logos Biosystems designed a reusable slide (LUNA™ Reusable Slide, L12008) to be used with the LUNA™ family of automated cell counters. Here, we compare the cell counting results of the LUNA™ Reusable Slide, a hemocytometer, and disposable slides.

MATERIALS AND METHODS

Comparing cell counting accuracy of the LUNA™ Reusable Slide and a hemocytometer

To determine the accuracy of cell counts done with the LUNA™ Reusable Slide, cell counts were compared to those obtained manually with a hemocytometer. Cells were serially diluted and mixed with an equal volume of 0.4% trypan blue stain. 10 µL of the mixture was loaded into the LUNA™ Reusable Slide and a glass hemocytometer with Neubauer counting grids (Marienfeld). To obtain cell concentrations with the glass hemocytometer, the hemocytometer was imaged with the iRiS™ Digital Cell Imaging System and the cells within the four corner squares of the Neubauer counting grids were counted. Cell samples in the LUNA™ Reusable Slide were counted with the LUNA-II™ Automated Cell Counter. All experiments were performed in triplicate.

Evaluating cell counting accuracy of the LUNA™ Reusable Slide with brightfield and fluorescence automated cell counters

The performance of the LUNA™ Reusable Slide for brightfield and fluorescence cell counting was evaluated by using two stains, trypan blue and acridine orange/propidium iodide (AO/PI), respectively. For brightfield counting, cells mixed 1:1 with trypan blue were loaded into the LUNA™ Reusable Slide or disposable slides and counted with the LUNA™, LUNA-FL™, and LUNA-II™ automated cell counters. For fluorescence cell counting, 2 µL AO/PI was mixed with 18 µL cell suspension and 10 µL was loaded into the LUNA™ Reusable Slide or disposable slides and counted with the LUNA-FL™ Automated Fluorescence Cell Counter. All experiments were performed in triplicate.

RESULTS

Cell counting accuracy of the LUNA™ Reusable Slide

To determine the cell counting accuracy of the LUNA™ Reusable Slide, the total cell concentration results obtained with the LUNA™ Reusable Slide were compared to those obtained manually with the hemocytometer. The LUNA™ Reusable Slide results were highly correlated (R2 > 0.999) with the hemocytometer results at the tested concentrations (Fig. 1). The cell concentration data for the LUNA™ Reusable Slide were more consistent at higher concentrations (>2 x 106 cells/mL) than the hemocytometer. The fact that the LUNA-II™ counts a larger area within the LUNA™ Reusable Slide than was manually counted with the hemocytometer may account for the difference.

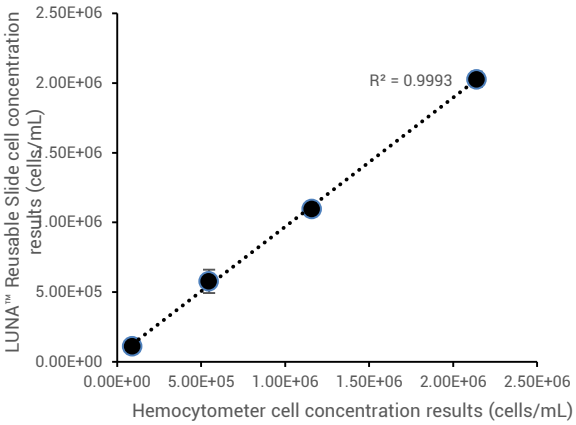


Fig 1. Comparison of the cell count results of the LUNA™ Reusable Slide and a hemocytometer.

Cell counting performance of the LUNA™ Reusable Slide for brightfield counting

To assess the compatibility of the LUNA™ Reusable Slide for automated brightfield counting, cells stained with trypan blue were loaded into the LUNA™ Reusable Slide and disposable slides and counted with the LUNA™, LUNA-II™. As shown in Fig. 2, there was no significant difference in the total, live, or dead cell concentrations among the slides or devices.

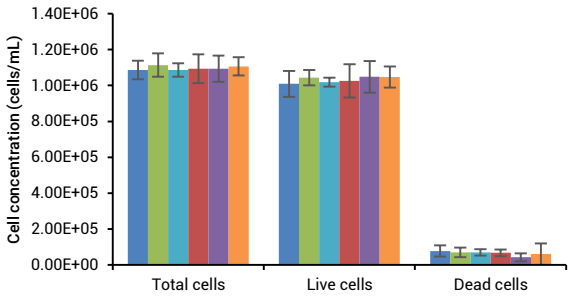
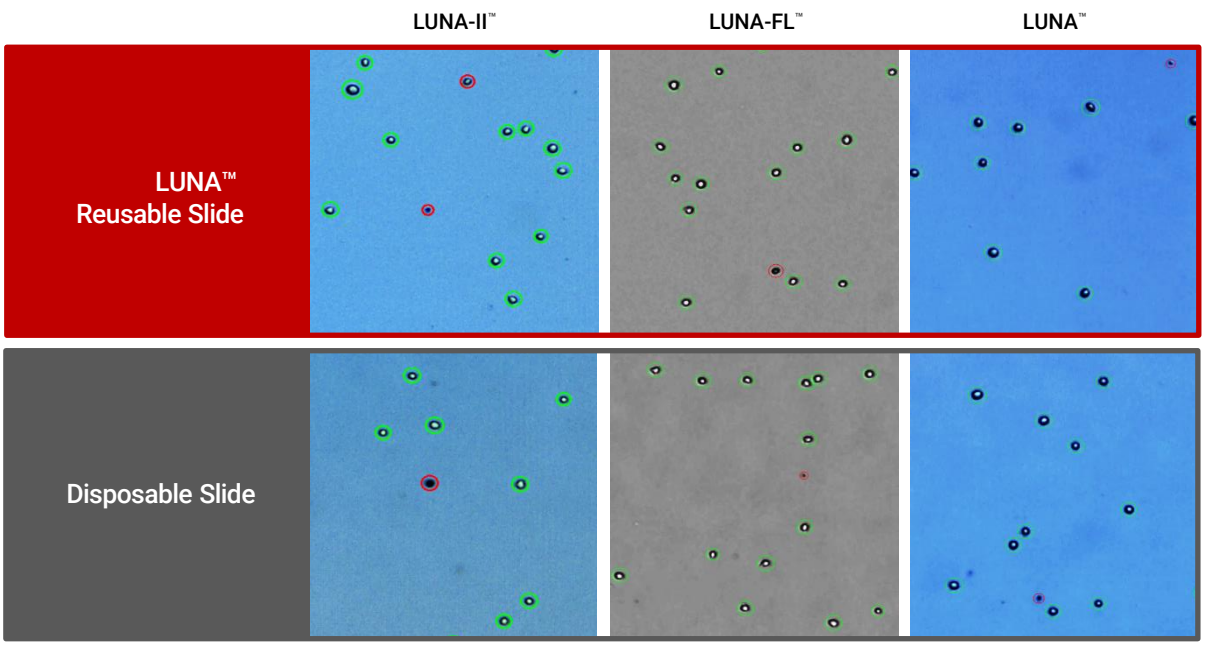


Fig. 2. Compatibility of the LUNA™ Reusable Slide with automated cell counters by trypan blue staining. (Top) Cell images captured and tagged by the LUNA™ family of automated cell counters; (bottom) cell counting results using the LUNA™ Reusable Slide and disposable slides.



RESULTS

Cell counting performance of the LUNA™ Reusable Slide for fluorescence staining

To assess the compatibility of the LUNA™ Reusable Slide for automated fluorescence counting, cells stained with AO/PI were counted with the LUNA-FL™. There were no significant differences in any of the parameters (Fig. 3).

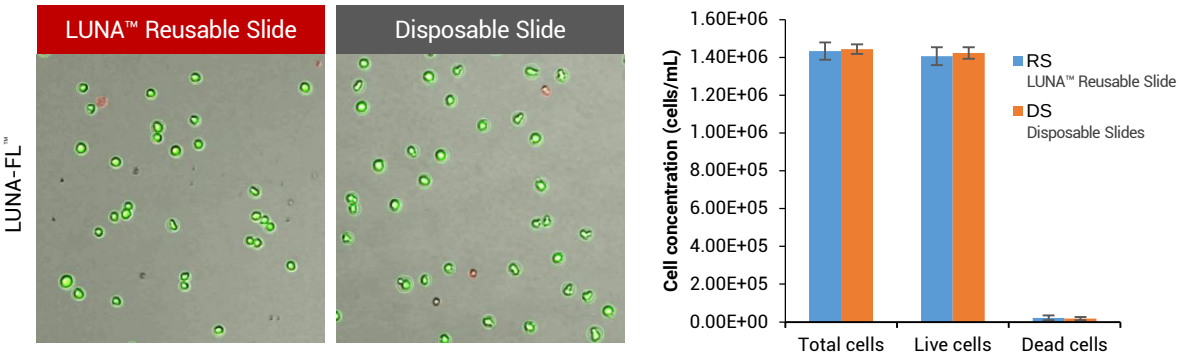


***Fig. 3**. Compatibility of the LUNA™ Reusable Slide with automated cell counters by AO/PI staining. (Top) Cell images captured by the LUNA-FL™; (bottom) cell counting results using the LUNA™ Reusable Slide and disposable slides.*

CONCLUSIONS

Automated cell counting with the LUNA™ Reusable Slide is a good alternative to the hemocytometer for the following re asons:

- No learning curve – similar to using a hemocytometer
- Reusable and cost-effective
- Accurate cell counting results
- Removal of user variability and time consuming calculations
- Compatible with autofocused automated counting (with the LUNA-II™ and LUNA-II YF™)

REFERENCES

Tucker KG, Chalder S, Al-Rubeai M, Thomas CR. Measurement of hybridoma cell number, viability, and morphology using fully automated image analysis. Enzyme Microb Technol. 16:29–35. (1994)

- **Ordering Information**

Automated Cell Counters			
Brightfield	L10001	LUNA™ Automated Cell Counter	1 unit
	L40001	LUNA-II™ Automated Cell Counter (with built-in printer)	1 unit
	L40002	LUNA-II™ Automated Cell Counter (without printer)	1 unit
Fluorescence	L20001	LUNA-FL™ Automated Fluorescence Cell Counter	1 unit
	L30001	LUNA-STEM™ Automated Fluorescence Cell Counter	1 unit
	L50001	LUNA-II YF™ Automated Yeast Cell Counter	1 unit
Slides			
Reusable	L12008	LUNA™ Reusable Slide	1 unit
	L12010	LUNA™ Reusable Slide Coverslips	10 units
Disposable	L12001	LUNA™ Cell Counting Slides, 50 Slides	1 box
	L12002	LUNA™ Cell Counting Slides, 500 Slides	10 boxes
	L12003	LUNA™ Cell Counting Slides, 1000 Slides	20 boxes
	L12005	PhotonSlide™, 50 Slides	1 box
	L12006	PhotonSlide™, 500 Slides	10 boxes
	L12007	PhotonSlide™, 1000 Slides	20 boxes
Stains			
Brightfield	T13001	Trypan Blue Stain, 0.4%	2 x 1 mL
	L13002	Erythrosin B Stain	2 x 1 mL
Fluorescence	F23001	Acridine Orange/Propidium Iodide Stain	2 x 0.5 mL
	F23002	Acridine Orange Stain	2 x 0.5 mL
	F23003	Propidium Iodide Stain	2 x 0.5 mL
Beads			
Brightfield	B13101	LUNA™ Standard Beads	2 x 1 mL
Fluorescence	F23102	LUNA™ Fluorescence Calibration Beads	1 x 0.5 mL

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