The Counting Accuracy of the LUNA-FX7™ Automated Cell Counter

INTRODUCTION

The need for accurate cell counts underpins advances in biotechnology. Cell counts are used in every phase of biotechnological activity, from cell passage determinations to more sophisticated bioassay, and single-cell sequencing analyses to cell therapeutic dosing determinations[1,2]. Accurate and consistent cell count ensures quality reliability in downstream procedures, while inaccurate and inconsistent cell counts adversely depreciate downstream processes. We present the LUNA-FX7™ is the newest member of the LUNA™ Automated Cell Counter family. With state-of-the-art optics, an improved counting algorithm, and precision autofocus, the LUNA-FX7™ was designed to meet the gamut of cell counting needs. Thus, the LUNA-FX7™ has augmented functionality in both brightfield and dual fluorescent cell counting modes. Here, we demonstrated the accuracy of cell viability with K562 cells and evaluated intra- and inter-instrument variability of the LUNA-FX7™ with calibration beads.

MATERIALS AND METHODS

Healthy K562 cells of 2 x 10^6 cells/mL were prepared, and half of the cells were heat-killed at 95°C for 30 minutes to obtain dead cells. Five different viability of the cells were prepared by mixing live and dead cells. The cells were then counted in both brightfield and dual fluorescent modes using Trypan Blue (Cat# T13101) and Acridine Orange/Propidium Iodide (AO/PI) (Cat# F23001), respectively. For assessing intra- and inter-device variability, counts were performed using green and red fluorescent beads with a predetermined concentration. Seven different LUNA-FX7™ devices were used to assess inter-device deviation. The samples were prepared by 1:1 GF and RF Calibration Bead mixture from the LUNA-FX™ Calibration Beads Kit (Cat# F73101) to make the viability rate 50% and loaded on a PhotonSlide™ (Cat# L12005).

RESUITS

The Reproducibility and Accuracy of K562 Cell Counting

Often, the accuracy and reliability of counters may decline due to some tricky viability of dead cells, which can prevent an accurate assessment. We prepared K562 cells of the expected total with the final concentration of 2 x 10^6 cells/mL of 5 different live/dead cell combinations (Figures 1 and 2). In both brightfield and fluorescence counting modes, the LUNA-FX7™ precisely determined the viabilities and concentrations across all the samples accordingly.

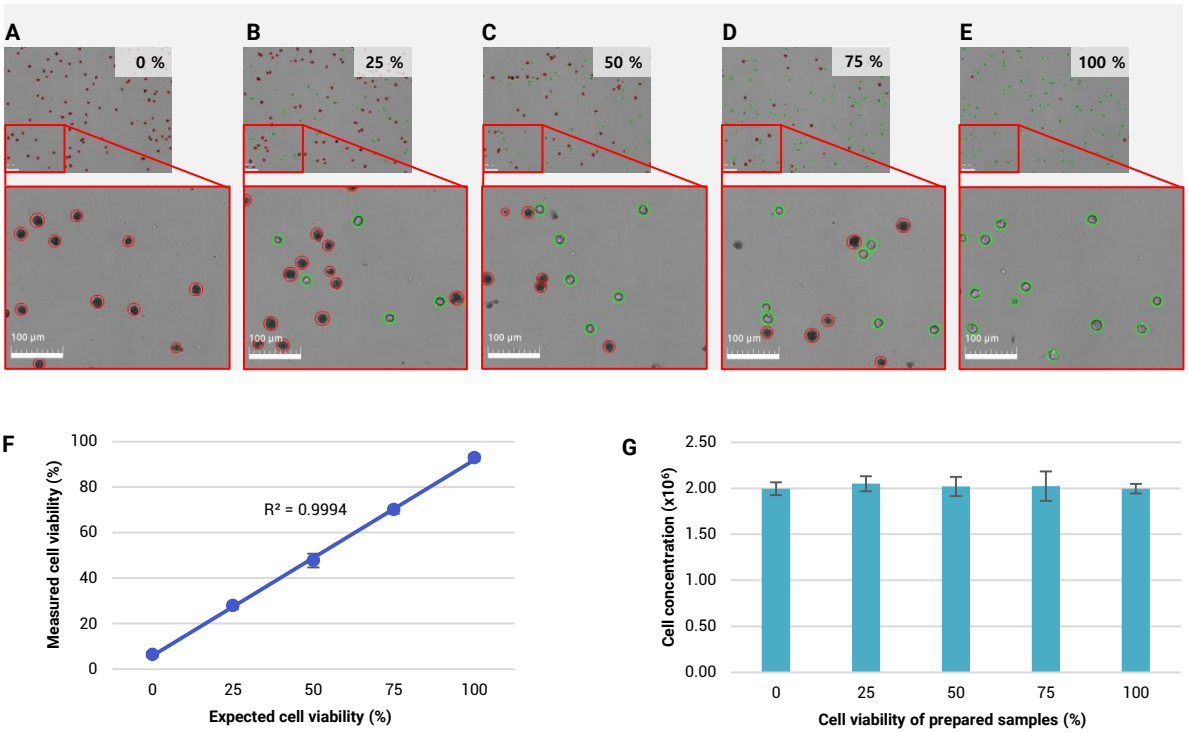

Figure 1. *The linearity of the LUNA-FX7™ in brightfield counting. Cells were counted in Bightfield Cell Counting & Viability mode using Trypan blue staining. (A-E) Tagged images of 0, 25, 50, 75 and 100% viability. (F) Measured viability of K562 plotted against the expected viability. (G) Constancy of the total cell concentrations at five different viability samples.*

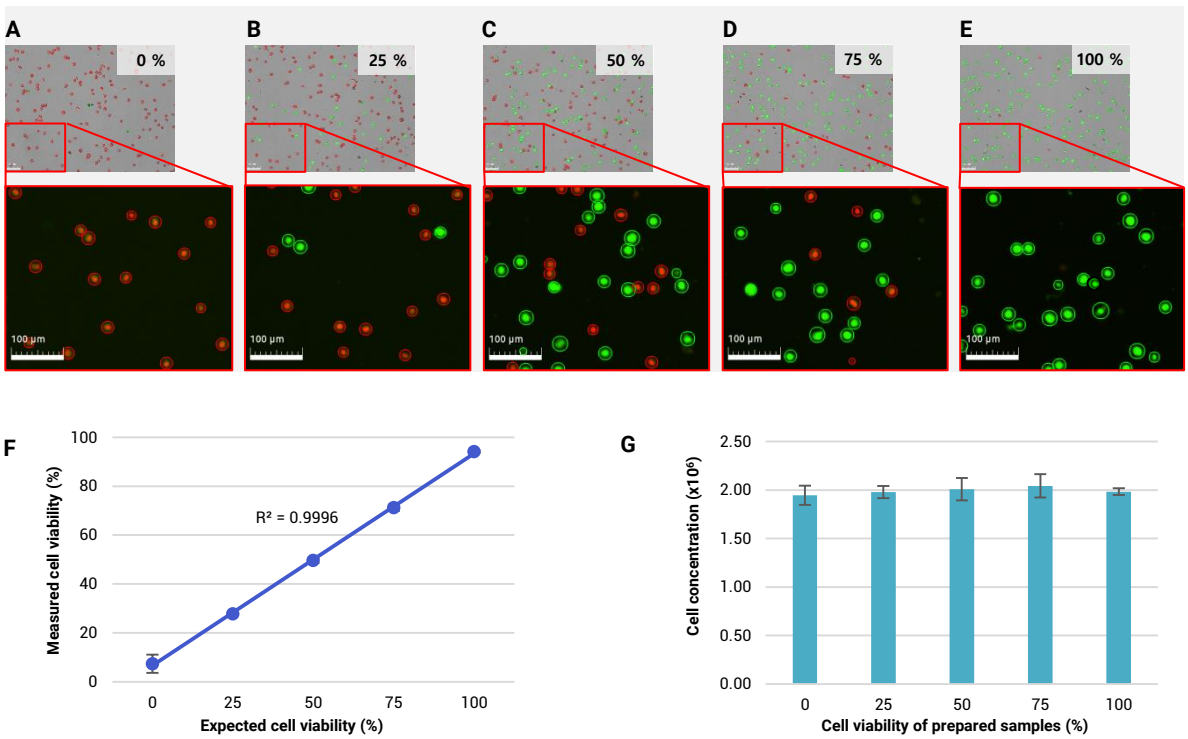

Figure 2. *The linearity of the LUNA-FX7™ in dual fluorescent counting. Cells were counted in Fluorescence Cell Counting mode using AO/PI dye. (A-E) Tagged images of 0, 25, 50, 75 and 100% viability, (F) Measured viability of K562 plotted against expected viability. (G) Measured total cell concentrations at five different viability samples are steady and not significant.*

Intra- and Inter- Device Deviation of the LUNA-FX7™ Automated Cell Counter

Counting reproducibility is essential for downstream applications of cultured cells. Demonstrating the consistency and reproducibility of the LUNA-FX7™, which provides reliable results, two different assessments were achieved. First, intra-deviation was determined by the repeated counting of the LUNA-FX™ Calibration Beads Kit (Cat# F73101): 10 µl of a 1:1 GF and RF Calibration Bead mixture was counted 5 times each across 4 independent devices (Table 1). Second, inter-device deviation was measured by counting four independent bead slides across seven different LUNA-FX7™ devices (Table 2). Both the intra- and inter-device evaluation of the LUNA-FX7™ reveals excellent counting reproducibility. In specifics, the intra-device variability for all four devices was 1% or less for cell concentration, viability, and cell size determination (Figure 3), and the inter-device variability was less than 2.02% for all parameters in 7 different devices.

Table 1. *Intra-device variability of the LUNA-FX7™*

LUNA-FX™ Calibration Beads, prep #1

Repeat	Concentration(/ml)	Viability(%)	Average size(µm)
1	1.00E+06	53.8	11.5
2	9.95E+05	53.7	11.5
3	9.97E+05	54.0	11.4
4	9.97E+05	53.9	11.4
5	1.01E+06	54.2	11.4
Average	1.00E+06	53.9	11.4
St. Dev.	5.97.E+03	0.2	0.1
CV(%)	0.60%	0.36%	0.48%

LUNA-FX™ Calibration Beads, prep #2

Repeat	Concentration(/ml)	Viability(%)	Average size(µm)
1	1.01E+06	54.6	11.5
2	1.00E+06	54.3	11.5
3	1.01E+06	53.1	11.6
4	9.92E+05	53.9	11.5
5	1.01E+06	54.4	11.5
Average	1.00E+06	54.1	11.5
St. Dev.	8.17.E+03	0.6	0.0
CV(%)	0.81%	1.10%	0.39%

LUNA-FX™ Calibration Beads, prep #3

Repeat	Concentration(/ml)	Viability(%)	Average size(µm)
1	1.00E+06	53.8	11.5
2	9.87E+05	54.0	11.6
3	9.92E+05	54.1	11.6
4	9.94E+05	54.5	11.8
5	1.01E+06	53.9	11.6
Average	9.97E+05	54.1	11.6
St. Dev.	8.82.E+03	0.3	0.1
CV(%)	0.89%	0.50%	0.94%

LUNA-FX™ Calibration Beads, prep #4

Repeat	Concentration(/ml)	Viability(%)	Average size(µm)
1	9.87E+05	54.0	11.6
2	1.01E+06	54.6	11.6
3	9.97E+05	54.1	11.5
4	1.00E+06	54.6	11.6
5	9.88+05	53.7	11.6
Average	9.99E+05	54.2	11.6
St. Dev.	9.47.E+03	0.4	0.0
CV(%)	0.95%	0.73%	0.39%

Table 2. *Inter-device variability of the LUNA-FX7™*

LUNA-FX™ Calibration Beads, prep #1

Repeat	Concentration(/ml)	Viability(%)	Average size(µm)
1	1.05E+06	51.1	11.1
2	1.04E+06	51.1	11.1
3	1.05E+06	52.1	11.2
4	1.05E+06	51.5	11.2
5	1.06E+06	52.6	11.2
6	1.02E+06	51.3	11.0
7	1.04E+06	51.2	11.3
Average	1.04E+06	51.56	11.16
St. Dev.	1.27.E+04	0.58	0.10
CV(%)	1.22%	1.12%	0.87%

LUNA-FX™ Calibration Beads, prep #2

Repeat	Concentration(/ml)	Viability(%)	Average size(µm)
1	1.08E+06	49.5	11.0
2	1.07E+06	50.3	11.2
3	1.08E+06	50.2	11.1
4	1.07E+06	50.6	11.1
5	1.06E+06	49.8	11.4
6	1.06E+06	50.2	11.0
7	1.08E+06	49.2	11.4
Average	1.07E+06	49.97	11.17
St. Dev.	9.00.E+03	0.49	0.17
CV(%)	0.84%	0.99%	1.53%

LUNA-FX™ Calibration Beads, prep #3

Repeat	Concentration(/ml)	Viability(%)	Average size(µm)
1	9.82E+05	50.9	11.2
2	1.00E+06	50.3	11.0
3	9.78E+05	51.1	11.2
4	9.98E+05	51.3	11.1
5	9.89E+05	52.1	11.2
6	9.72E+05	52.4	11.1
7	9.77E+05	51.9	11.2
Average	9.85E+05	51.43	11.14
St. Dev.	1.08.E+04	0.74	0.08
CV(%)	1.10%	1.44%	0.71%

LUNA-FX™ Calibration Beads, prep #4

Repeat	Concentration(/ml)	Viability(%)	Average size(µm)
1	1.05E+06	52.5	11.1
2	1.04E+06	52.8	11.0
3	1.05E+06	53.0	11.2
4	1.01E+06	50.8	11.0
5	1.01E+06	51.3	11.2
6	1.02E+06	52.9	11.1
7	1.00E+06	50.6	11.5
Average	1.03E+06	51.99	11.16
St. Dev.	2.07.E+04	1.05	0.17
CV(%)	2.02%	2.02%	1.54%

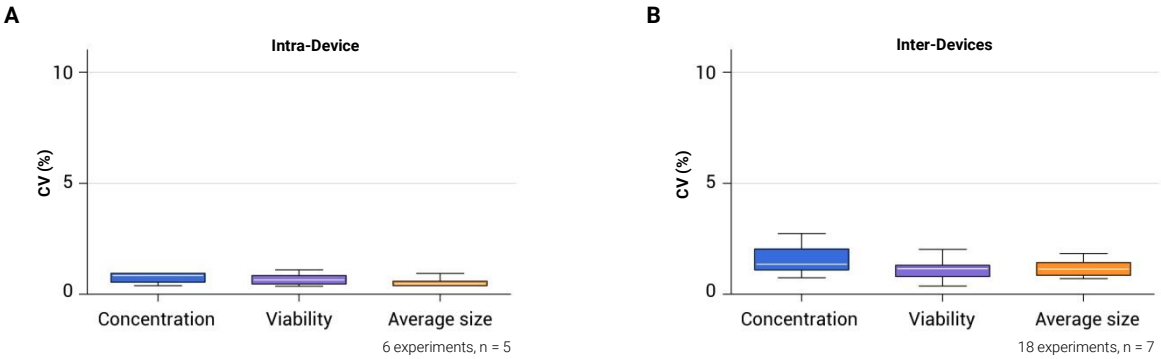

Figure 3. *The intra-device and inter-device reproducibility of the LUNA-FX7™. The coefficient values (CV%) of the intra-device and inter-device measurements result in 3% or lower.*

CONCLUSION

The LUNA-FX7™ Automated Cell Counter provides exceptionally accurate and consistent values for cell concentration, viability, and average size, regardless of device and operators. The intra- and inter-device variabilities are minimal, providing confidence in the reproducibility and reliability in the data from one to multiple LUNA-FX7™s based on the needs of research laboratories and facilities.

REFERENCES

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[2]Johnston G. Automated handled instrument improves counting precision across multiple cell lines. BioTechniques. 2010;48:325-7.