Assessment of an Automated Cell Counting Approach in the Biobanking Workflow of Parasitic Protists

2840

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BACKGROUND

The accessibility of reference strains available in biorepositories is critical to studies aimed at elucidating the mechanisms used by highly adapted parasites to block or subvert host processes and for therapeutic or vaccine development. The biobanking workflow of parasitic protists housed at the American Type Culture Collection (ATCC) includes a variety of quality control tests, including among others, measurements of cell densities of stocks, verification of viability and purity, and phenotypic and genotypic analyses. Automated counters have become the method of choice among various biorepositories for cell counting over traditional manual methods that use hemacytometers and light microscopy. Importantly, automation has been primarily applied to biobanks of mammalian cell lines, while little if any research has been conducted in the quality control of parasitic protist stocks. The present study evaluated the accuracy of the Cellometer Spectrum 10X system compared to the hemocytometer chamber in the counting of *Leishmania donovani* and *Giardia lamblia* cells as part of biobanking test procedures. After evaluating the accuracy of the automated cell counting method, we assessed the performance of the assay to determinations of parasite cell viability. Based on our data, we provide recommendations on using the automated cell count method as a plausible procedure in the quality control of parasitic protist stocks available in biorepositories.

METHODS

Parasite strains. The following strains were obtained from the BEI Resources Repository, NIAID, NIH (www.beiresources.org): *Leishmania donovani* 1S2D (BEI NR-50182) and *Giardia lamblia* WB C6 (BEI NR-9706). Cultures of *Leishmania* were incubated at 25ºC in Medium 199 (ThermoFisher No. 11150059) supplemented with 10% heat-inactivated FBS (ATCC® 30-2021) and 10 µg/ml of hemin (Sigma No. H9039). Cultures of *Giardia* were incubated at 35ºC in ATCC® medium 2155.

Expansion of cultures. *Leishmania* cultures were seeded at a density of 1 x 10^6 cells/mL in 25 cm^2 T-flasks and incubated at 25ºC for 3 days. *Giardia* cultures were seeded at densities of 1 x 10^6 cells/mL and incubated at 35ºC for 3 days in 125 mm glass test tubes. To perform the cell counting procedures, parasite cultures were collected and centrifuged at 800 *g* for 10 min. Cell pellets were resuspended in 1 mL of culture medium and processed as described below.

Manual Cell counts. The concentrations of parasite cell suspensions were initially determined by the routine hemocytometer method. Briefly, suspensions were diluted 1:10 in culture medium and 10 µl of sample were loaded in the chamber. Cell counts were performed in each of the four large squares. The cell concentrations were determined by averaging the four readings and calculated as follows: Cells/mL = (Average of 4 squares) x (dilution factor) x (1 x 10^4).

Automated Cell Counts. Ten µl of cell suspensions were mixed with 10 µl of acridine orange/propidium iodide (AO/PI) stain. Samples were loaded in SD100 slides and read in the Cellometer Spectrum 10X instrument. Assay parameters for automated *Leishmania* and *Giardia* cell counts were set with the Spectrum 10X software using the following parameters: cell diameter=3.0-25.0 µm, roundness value=0.10, and contrast enhancement=0.40.

Comparison of manual versus automated cell counts. The comparison of cell counts between the two methods was performed by two operators (OP 1 and OP 2) as shown in Figure 1. The data from three experiments were statistically analyzed by average, standard deviation, and coefficient of variation (CV) percentages within each technician (intra-operator) and between technicians (inter-operator). Where indicated, viability counts between manual and automated methods were also performed by Trypan Blue dye exclusion and live/dead (AO/PI) fluorescence analysis, respectively.

RESULTS

Fig. 1. Experimental Approach

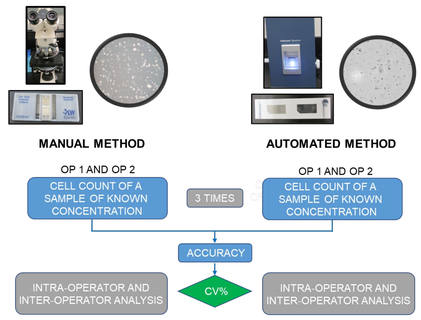
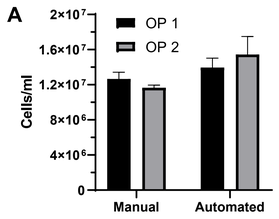


Fig. 1. Comparison of manual and automated cell counts using parasite cultures. The concentrations of the samples were initially quantified using the routine hemocytometer/manual method. Both operators performed total cell counts to evaluate the accuracy of the Cellometer Spectrum compared to the hemocytometer chamber. The test was performed three times under the same operating conditions by OP 1 and OP 2 and intra- and inter-operator coefficient of variation (CV%) was calculated.

Fig. 2. Evaluation of Automated Cell Counts Using Cultures of *Leishmania*

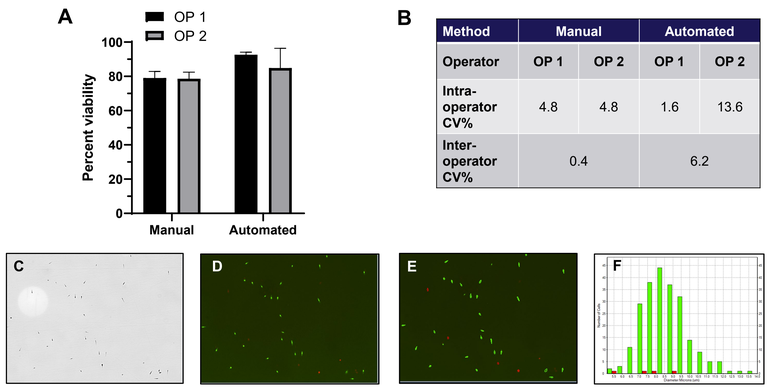


B

Method	Manual		Automated	
Operator	OP 1	OP 2	OP 1	OP 2
Intra-operator CV%	6	2.5	7.5	13.3
Inter-operator CV%	5.8		7	

Fig. 2. Comparison of manual and automated cell counts using cultures of *Leishmania*. The cell concentration of the *Leishmania* culture was initially adjusted to 1 x 10^7 cells/mL using the routine hemocytometer/manual method. Both operators performed manual cell counts and automated cell counts in triplicate. The data were analyzed by calculating the means±standard deviations **(A)** and coefficient of variation (CV) percentages **(B)** within each technician (intra-operator CV%) and between technicians (inter-operator CV%).

Fig. 3. Evaluation of *Leishmania* Cell Viability by the Automated Method



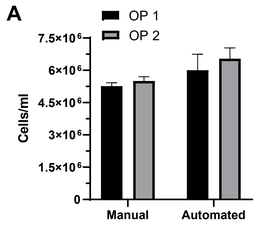
B

Method	Manual		Automated	
Operator	OP 1	OP 2	OP 1	OP 2
Intra-operator CV%	4.8	4.8	1.6	13.6
Inter-operator CV%	0.4		6.2	

Fig. 3. Estimation of *Leishmania* cell viability by manual and automated methods. *Leishmania* cell viability counts by the manual and automated methods were performed by Trypan Blue dye exclusion and live/dead (AO/PI) fluorescence analysis, respectively. The data from OP 1 and OP 2 were collected from three experiments and analyzed by calculating the means±standard deviations **(A)** and coefficient of variation (CV) percentages **(B).** Brightfield image **(C)**, live/dead cell fluorescent image **(D),** live/dead fluorescent cell count image **(E)**, and cell diameter histogram **(F)** were obtained with the Spectramax software from a representative experiment of three performed.

RESULTS

Fig. 4. Evaluation of Automated Cell Counts Using Cultures of *Giardia*

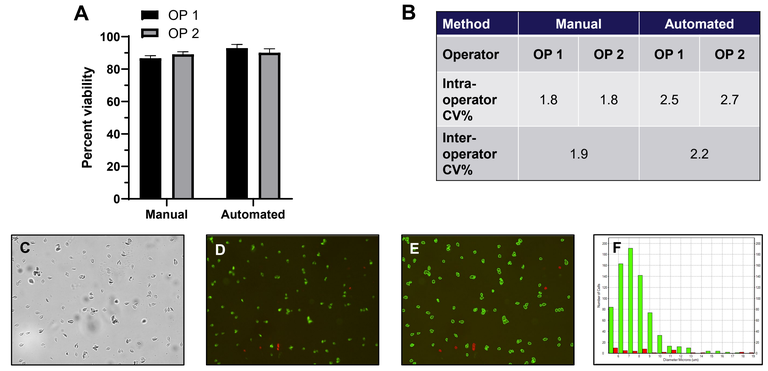


B

Method	Manual		Automated	
Operator	OP 1	OP 2	OP 1	OP 2
Intra-operator CV%	2.9	3.6	12.4	7.6
Inter-operator CV%	3.1		6.1	

Fig. 4. Comparison of manual and automated cell counts using cultures of *Giardia*. The cell concentration of the *Giardia* culture was initially adjusted to 5 x 10^6 cells/mL using the routine hemocytometer/manual method. Both operators subsequently performed manual cell counts and automated cell counts in triplicate. The data were analyzed by calculating the means±standard deviations **(A)** and coefficient of variation (CV) percentages **(B)** within each technician (intra-operator CV%) and between technicians (inter-operator CV%).

Fig. 5. Evaluation of *Giardia* Cell Viability by the Automated Method



B

Method	Manual		Automated	
Operator	OP 1	OP 2	OP 1	OP 2
Intra-operator CV%	1.8	1.8	2.5	2.7
Inter-operator CV%	1.9		2.2	

Fig. 5. Estimation of *Giardia* cell viability by manual and automated methods. *Giardia* cell viability counts by the manual and automated methods were performed by Trypan Blue dye exclusion and live/dead (AO/PI) fluorescence analysis, respectively. The data from OP 1 and OP 2 were collected from three experiments and analyzed by calculating the means±standard deviations **(A)** and coefficient of variation (CV) percentages **(B).** Bright field image **(C)**, live/dead cell fluorescent image **(D),** live/dead fluorescent cell count image **(E)**, and cell diameter histogram **(F)** were obtained with the Spectramax software from a representative experiment of three performed.

SUMMARY

- The present study examined the feasibility of using an automated cell count method as a quality control test in the biobanking workflow of parasite cultures.
- Intra-operator total cell count CV% fell in the range of 7 to 16% for the automated method compared to 2 to 6% for the manual method. In general, total cell counts were 16-25% higher with the automated method.
- Use of the Cellometer offers benefits such as speed, use of large-scale samples, and disposable slides. However, routine use in protist biobanking requires further optimization when handling parasite cells of diverse sizes and morphologies.

ACKNOWLEDGEMENTS

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