

Assaying Sf9 Insect Cells with Guava® ViaCount® Flex: A Validation Study



TECHNICAL NOTE

ABSTRACT The Guava ViaCount assay provides cell counts and viability data quickly and easily for a variety of mammalian cell lines, both suspension and adherent. However, the Guava ViaCount reagent is incompatible with some non-mammalian cell lines, in particular, insect cells, which are becoming increasingly important for the expression of transfected proteins. The Guava ViaCount Flex reagent was developed to address the special needs of non-mammalian cell lines. Guava ViaCount Flex was optimized and validated using Sf9 insect cells that were adapted to suspension culture in serum-free medium. Results from the ViaCount Flex assay on the Guava Personal Cell Analysis (PCA™) system were compared to cell counts and viability results obtained using trypan blue and a hemacytometer. Good correspondence for cell count and viability results was seen for the two methods over a broad range of cell densities (about 10^4 to 6×10^5 cells/mL) and culture conditions (about 65% - 90%). Assay results were highly reproducible, with % CV for replicate total cell counts from three different operators ranging from 5% - 14% for the Guava ViaCount assay vs. 16% - 41% for hemacytometer counting. The % CV for replicate viability measurements of Sf9 cultures from three (3) different operators was 2% - 4% for the Guava ViaCount assay vs. 6% - 14% for hemacytometer counting of trypan blue-stained cells. Instrument-to-instrument precision was <5% CV for cell counts and <2% CV for viability measurements. Cell counts were linear over a broad range of concentrations (10^4 to $>5 \times 10^5$ cells/mL). The viability of Sf9 cells was unchanged for at least 45 minutes after staining with Guava ViaCount Flex reagent.



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Guava Technologies

Assaying Sf9 Insect Cells with Guava® ViaCount® Flex: A Validation Study

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Abstract

The Guava ViaCount assay provides cell counts and viability data quickly and easily for a variety of mammalian cell lines grown in suspension cultures. However, the Guava ViaCount reagent is incompatible with some non-mammalian cell lines, in particular, insect cells, which are becoming increasingly important for the expression of transfected proteins. The Guava ViaCount Flex reagent was developed to address the special needs of non-mammalian cell lines. Guava ViaCount Flex was optimized and validated using Sf9 insect cells that were adapted to suspension culture in serum-free medium. Results from the ViaCount Flex assay on the Guava Personal Cell Analysis (PCA™) system were compared to cell counts and viability results obtained using trypan blue and a hemacytometer. Good correspondence for cell count and viability results was seen for the two methods over a broad range of cell densities (about 10^4 to 6×10^5 cells/mL) and culture conditions (about 65% - 90%). Assay results were highly reproducible, with % CV for replicate total cell counts from three different operators ranging from 5% - 14% for the Guava ViaCount assay vs. 16% - 41% for hemacytometer counting. The % CV for replicate viability measurements of Sf9 cultures from three (3) different operators was 2% - 4% for the Guava ViaCount assay vs. 6% - 14% for hemacytometer counting of trypan blue-stained cells. Instrument-to-instrument precision was <5% CV for cell counts and <2% CV for viability measurements. Cell counts were linear over a broad range of concentrations (10^4 to $>5 \times 10^5$ cells/mL). The viability of Sf9 cells was unchanged for at least 45 minutes after staining with Guava ViaCount Flex reagent.

Introduction

Insect cells are becoming important for recombinant protein production. They provide advantages over both bacterial and mammalian cell systems. Bacterial cells, when transfected with mammalian gene sequences, often generate proteins with inadequate glycosylation patterns or inferior antigenic components. Mammalian cell systems require controlled environments for maximal growth and expensive media. In contrast, insect cells grow at room temperature, are robust, and the expressed proteins often are glycosylated in a manner that increases their immune response. For this reason, insect expression systems have gained increasing utility in the production of commercial vaccines. Insect cells also play a key role in studies of insect pathogens and disease states.

Researchers use a number of different insect cell lines for research and commercial purposes. Many biotechnologists favor the Sf9 and Sf21 cell lines, derived from the pupal ovarian tissue of the fall army worm, *Spodoptera frugiperda*, by scientists at the U. S. Department of Agriculture Insect Pathology Laboratory in Beltsville, Maryland. These two lines are used extensively for the isolation and propagation of recombinant baculovirus stocks and recombinant proteins. Researchers in drug discovery are using Sf9 to express estrogen receptors, viral capsid proteins, opioid receptors, and other proteins for further characterization.^{1,2}

Other insect cell lines include S2, which lends itself to heterologous protein expression. Derived from *Drosophila melanogaster* embryos, S2 grows easily in suspension. Researchers at the Boyce Thompson Institute for Plant Research in Ithaca, New York, created the High Five™ cell line, called BTI-TN-5BI-4,

from embryonic ovarian cells of the cabbage looper, *Trichoplusia ni*.³ High Five grows readily in suspension, and produces high yields of transfected proteins.

In bioprocess development and manufacturing, obtaining accurate and reproducible cell counts and viability assessments are important in monitoring and maintaining cultures for optimal productive conditions. The Guava ViaCount assay provides these results quickly and easily, with higher reproducibility than manual hemacytometer counts. Guava ViaCount distinguishes between viable and non-viable cells based on the differential permeability of DNA-binding dyes. One dye is membrane permeable, and stains all nucleated cells. The fluorescent signal from this nuclear staining dye is detected by photomultiplier tube 2 (PM2) in the Guava PCA instrument. The other dye only penetrates cells with compromised membrane integrity (i.e., non-viable cells) and is detected by photomultiplier tube 1 (PM1). Events are counted if they emit a nucleated cell fluorescent signal, and the forward light scatter intensity is appropriate for that of a particle the size of a cell. Non-viable cells emit fluorescent signals from the dead cell stain.⁴ Absolute cell counts are obtained on the Guava PCA system by knowing the exact sampling volumes. The cell suspension is drawn by positive displacement into the capillary flow cell of known dimensions at a precisely controlled rate for measured periods of time.

Because the Guava ViaCount assay was designed for mammalian cell systems, some characteristics of insect cells make them harder to analyze with the standard Guava ViaCount reagent. Although Sf9 cells can be assayed using Guava ViaCount reagent, the data must be obtained from the stained cells immediately after the 5-minute incubation period. Longer incubation times in Guava ViaCount reagent lead to loss of viability. Insect cells grow optimally at pH 6.0 to 6.5, rather than at the neutral pH preferred by mammalian cell systems, and insect cells prefer higher osmolarity than mammalian cells. Guava ViaCount Flex was designed to address these special needs of insect cells and other non-mammalian cell lines.

ViaCount Flex contains the same fluorescent DNA-binding dyes used in Guava ViaCount reagent, but in a highly concentrated formulation in DMSO. Guava

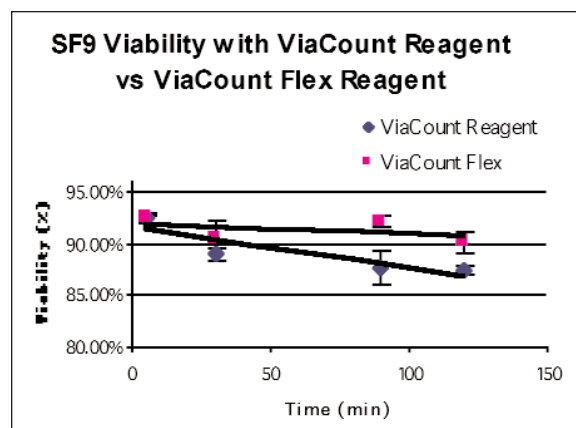


FIGURE 1: Post-stain stability of Guava ViaCount Flex vs ViaCount reagent on Sf9 cells. Sf9 cells were stained with Guava ViaCount Flex reagent and standard ViaCount reagent, according to instructions on the respective reagent product inserts. Cell count and viability measurements were taken using the Guava PCA system at periodic intervals over a 2 hour time course. The % Viability results over the time course are plotted. The viability of Sf9 cells in standard ViaCount reagent (blue diamonds) declines steadily over time, post staining, whereas the viability is stable with ViaCount Flex reagent (pink squares).

ViaCount Flex reagent allows the option to work with buffers or media that are more compatible for cell lines that lose viability in standard Guava ViaCount reagent. Figure 1 shows the change in viability of Sf9 cells incubated in Guava ViaCount reagent compared to ViaCount Flex over time. Stained cells were assayed on the Guava PCA system immediately after a 5-minute incubation, then at periodic time points for 120 minutes. As shown in Figure 1, viability steadily decreases in the Guava ViaCount reagent sample, from 92.5% to 87%. In contrast, Sf9 cell viability remains stable in the ViaCount Flex sample out to 90+ minutes in this experiment.

In addition, ViaCount Flex allows users to assay cell samples at low concentrations. Cell sample concentrations as low as 1×10^4 cells/mL can be assayed directly for cell count and viability. In contrast, cell counts cannot be performed accurately for samples at this low a concentration using Neubauer-type hemacytometers without first concentrating the cell sample, thereby introducing increased error in the measurement. ViaCount Flex requires just 200 μ L of the cell suspension (2000 cells from a sample at a concentration of 1×10^4 cells/mL) for accurate cell counts and viability assessments.

We have validated the ViaCount Flex assay on Sf9 cells, comparing count accuracy and precision measurements obtained on the Guava PCA system with manual hemacytometer counts using trypan blue exclusion. We characterized linearity of cell counts over a range of cell densities and culture viabilities, as well as post-stain sample stability. The results of our validation study are presented here.

Materials and Methods

Scope of validation study

This validation study involved comparing cell counts and viability assessments of Sf9 insect cells. For accuracy and precision studies, results from manual hemacytometer counts of trypan blue stained cells were compared to those obtained from ViaCount Flex stained cells run on the Guava PCA system using Cytosoft™ software. We performed additional precision studies: 1) instrument-to-instrument variability using three Guava PCA instruments, and 2) operator-to-operator variation with three laboratory personnel. In addition, the validation included sample linearity and characterization of post-stain sample stability.

Cell lines and culture conditions

Adherent Sf9 insect cells were adapted to serum-free growth in Sf-900 II Serum-Free Media (SFM) (GIBCO-Invitrogen Corp., Catalog No. 10902) for suspension growth. Cryopreserved Sf9 cells were obtained from the same vendor (Cat. No. 11496015). The cells were adapted to suspension growth in shake flask cultures. The Sf9 cultures were incubated at 28°C in non-CO₂, ambient air equilibrated incubators. Adaptation of the adherent culture to suspension growth took 1-3 weeks.

To obtain high viability cells, the cultures were split to a density of 3×10^5 cells/mL every 2 or 3 days with SF 900 II SFM. For low viability samples, cells were overgrown to a high density (approximately $2\text{-}3 \times 10^6$ cells/mL) and left at room temperature overnight to obtain cells of less than 25% viability. Low viability SF9 cells were spiked into healthy SF9 cells to obtain the desired viability and desired cell concentration the day the assay was performed.

Manual cell count and viability

A Neubauer-type hemacytometer (Hausser Scientific, Part No. 1492) was used to perform manual cell counts in combination with an epi-fluorescence microscope (Leica DM LB). Sf9 cell samples were stained with trypan blue (Sigma, Cat. No. T8154). Sf9 cells in SF 900 II SFM media were transferred to a 1.5 ml tube at a volume of 1.0 mL. Then 0.1 ml of 0.4% trypan blue solution (w/v) (Sigma, T8154) was added to the same tube. The sample was mixed thoroughly and left to stain for 15 minutes. With the coverslip in place, a pipette was used to transfer 10 µL of the trypan blue-stained cell suspension to each chamber of the hemacytometer. Cells were counted in the four corners and middle square of the hemacytometer, starting with one chamber. Non-viable cells stain blue, while viable cells appear unstained. Viable and non-viable cells were tallied separately. The procedure was repeated for the other chamber. Cell concentrations were calculated from hemacytometer counts.^{5,6} The following formula was used to calculate the number of viable cells per mL of culture: Number of viable cells $\times 10^4 \times 1.1$ (Dilution Factor). Viabilities were calculated as percentages: % Viable cells = [(Live cell count/Total cell count) $\times 100$]. Total cell count equaled the sum of the live cell count plus dead cell count.

Guava ViaCount Flex assay

The Guava ViaCount Flex assay was performed as described in the package insert for the product (Part No. 4600-0370). Briefly, ViaCount Flex (Catalog No. 4500-0110, Part No. 4000-0130) was diluted 1:2 with DMSO and mixed well to make a 0.5X ViaCount Flex reagent. Cell samples were mixed by vortexing, and then 200 µL of cell suspension were transferred to a 1.5 mL microcentrifuge tube and 2 µL of the 0.5X ViaCount Flex reagent was added. The sample was mixed until a homogenous pink color appeared, and no dark purple reagent was visible. The sample was incubated for 5 minutes at room temperature for dye equilibration, then the sample was acquired on the Guava PCA system.

Data Acquisition

Proper instrument performance was verified by running the Guava Check application with Guava Check

reagents (Catalog No. 4500-0020). Data were acquired on the Guava PCA system using CytoSoft software as described in the *Guava PCA User's Guide* and respective package inserts. Default instrument settings for the ViaCount application were used. Typically, 1000 events were collected per sample.

Cell count and viability correlation between ViaCount Flex and trypan blue exclusion assays. Seven (7) different Sf9 culture samples, at concentrations ranging from about 10^4 to 6×10^5 cells/mL and viabilities of 65% to 90%, were assayed using ViaCount Flex with the Guava PCA system and trypan blue exclusion with manual hemacytometer counting. For each culture, 9 replicate samples were stained with ViaCount Flex and data was acquired from each test sample twice, yielding $N = 18$ measurements. For manual counts, 6 replicate samples were stained with trypan blue and each test sample was counted once, as described above, yielding $N = 6$ measurements.

Count linearity study for the ViaCount Flex assay. Five (5) experiments were performed, using different Sf9 culture samples each time. For each experiment, a culture was diluted with Sf-900 II SFM to yield 5 test concentrations, ranging from 10^4 to 5×10^5 cells/mL. Five (5) replicate samples from each test concentration were stained with ViaCount Flex reagent, then run on the Guava PCA system. Data was acquired twice per sample, yielding $N = 10$ data points per concentration. For manual counts, three (3) replicate samples ($N = 3$) from each Sf9 test concentration were stained with trypan blue and counted on the fluorescence microscope using a hemacytometer.

The expected value was based on the trypan blue manual count of the undiluted Sf9 stock for each culture. Expected concentrations were calculated using this number and the known dilution factors used to prepare the different test concentrations.

Instrument Variability

Cells counts for three concentrations of Sf9 cells (about 10^4 , 1.5×10^5 , and 5×10^5 cells/mL) were performed on three different Guava PCA instruments. Ten (10) replicate samples from each test concentration were prepared for the ViaCount Flex assay as described previously. Data was acquired

using the same test samples on three different Guava PCA instruments. The default PM1 and PM2 voltage settings and forward scatter photodiode gain were used for each Guava PCA instrument. Thresholds and gate settings were optimized for each analysis. Data was acquired twice per sample, yielding $N = 20$ data points per concentration per instrument.

Operator Variability

Three (3) operators prepared replicate samples from seven (7) different Sf9 cultures independently. Each operator stained triplicate samples of each Sf9 culture with ViaCount Flex and duplicate samples with trypan blue. The Sf9 cultures were at concentrations ranging from 10^4 to 5×10^5 cells/mL and viabilities ranging from 65% to 90%. All samples were prepared and assayed by the three operators on the same day, using the same Guava PCA instrument and microscope. Each operator performed the ViaCount Flex assay, acquiring data from the triplicate samples twice ($N = 6$ data points per culture per operator). Each operator scored the trypan blue stained samples manually at the microscope ($N = 2$ measurements per culture per operator). Total cell counts and % viability results were derived.

Post-Stain Sample Stability

Four (4) different Sf9 cultures were obtained, with viabilities of 90+%, 70%, 50% and 20%. These cultures were diluted to yield three (3) different stock concentrations: 5×10^4 cells/mL (Low), 2.5×10^5 cells/mL (Medium), and 5×10^5 cells/mL (High). Triplicate (3) test samples were prepared from each of the twelve (12) cell stocks. Over a defined time course interval, the replicate test samples from each of the cell stocks were stained with ViaCount Flex reagent and run on the Guava PCA system immediately after staining. Data was acquired from each test sample twice at periodic intervals out to 4 hours post staining ($N = 6$ data points at each time point per cell stock).

Results and Discussion

Accuracy of cell counts and viability. We compared cell counts and viability assessments from manual hemacytometer counts using trypan blue to counts

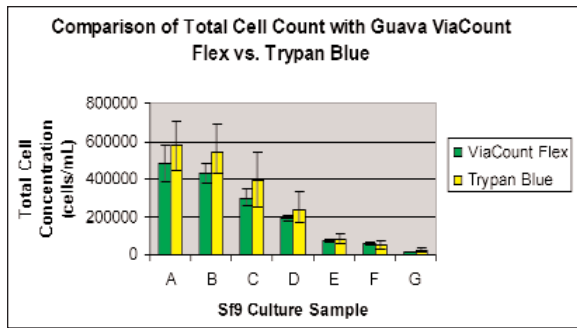


FIGURE 2: Comparison of total cell count results obtained using Guava ViaCount Flex on the Guava PCA system (green bars) and manual hemacytometer counts of trypan blue-stained samples (yellow bars). The seven (7) different Sf9 culture samples spanned a range of concentrations from about 1×10^4 to 6×10^5 cells/mL. The average value of replicate counts is shown by the bars (N = 18 for Guava ViaCount; N = 6 for Trypan Blue, per culture). The error bars represent the high and low range of the count values.

obtained using ViaCount Flex reagent and the Guava PCA system. Figure 2 shows the comparison of total cell count results for seven (7) different test cultures of Sf9 cells. Figure 3 shows the comparison of the % viability results for the same test cultures. The average values are plotted in each graph, with the error bars defining the range of values seen for replicate measurements. The seven culture samples had cell concentrations ranging from about 10^4 to 6×10^5 cells/mL with viabilities of about 65% to 90%.

In Figure 2, the average values for total cell count differ by 10%-25% for test cultures A – F, while the difference for culture G was about 40% when comparing the Guava PCA vs. manual counting. However, the range of total cell count results overlap between the two methods for all cultures (as noted by error bars) and there was no statistically significant difference between the total cell count values obtained using ViaCount Flex and the Guava PCA instrument vs. trypan blue manual counting ($p = 0.01$).

In Figure 3, the average % Viability values differed by 2% or less for all cultures except A and F when comparing ViaCount Flex vs. trypan blue exclusion. Indeed, there was no statistically significant difference between the viability results for these cultures ($p = 0.01$). However, there was an 8% difference between the two methods for culture A and a 15% difference for culture F, which were statistically sig-

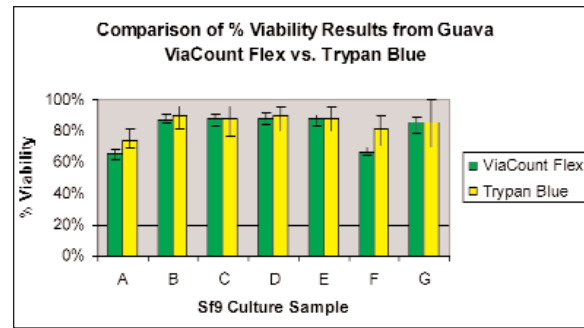


FIGURE 3: Comparison of % Viability results obtained using Guava ViaCount Flex on the Guava PCA system and manual hemacytometer counts of trypan blue-stained samples. The seven (7) different Sf9 culture samples spanned a range of viabilities from about 66% to 90%. The average value of replicates is shown by the bars (N = 18 for Guava ViaCount; N = 6 for Trypan Blue). The error bars represent the high and low range of replicate count values. Culture samples correspond to those shown in Figure 2.

nificant. The trypan blue exclusion assay showed higher viability than the ViaCount Flex assay for these two culture samples, which were of lower viability (about 65%) than the others (close to 90%). We have seen these results before with cultures at lower viability. Guava ViaCount reagent and ViaCount Flex allow the identification of apoptotic cells, distinct from healthy live cells and dead cells,⁷ whereas trypan blue does not. Many apoptotic cells do not stain blue and appear viable by trypan blue exclusion, leading to an overestimate of culture viability. Mascotti et al. reported seeing an overestimate of viability with trypan blue exclusion when comparing counts of bone marrow hematopoietic precursors done with acridine orange and propidium iodide (PI).⁸ Altman et al. reported seeing erroneous culture viabilities with trypan blue when comparing cells assayed with fluorescein diacetate and PI.⁹ Thus, the results obtained were not surprising and we feel the ViaCount Flex is providing a more realistic assessment of the culture viability than trypan blue exclusion.

Linearity of cell counts. The ViaCount Flex assay and manual counts for this linearity study were performed on five different Sf9 culture samples. Samples were prepared as described in the Materials and Methods section. Figure 4 shows typical results from one of the five experiments, using an Sf9 culture with about 75% viability. Averages of the total cell counts are plotted in Figure 4, with the error bars indicating the standard deviation of the replicate measure-

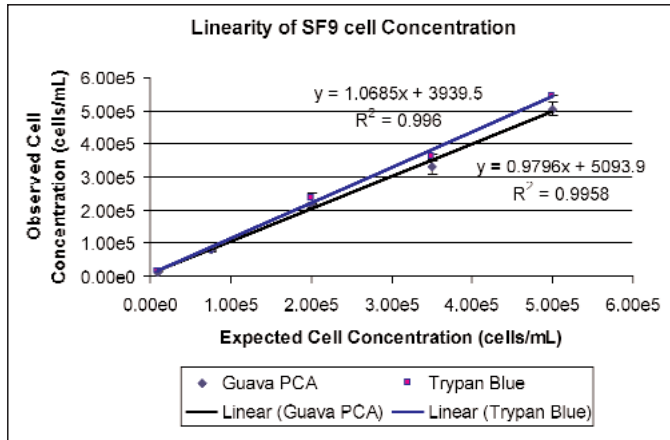


FIGURE 4: An aliquot from a single Sf9 culture was taken and used to prepare five (5) dilutions to span a range of cell concentrations from about 1×10^4 to 6×10^5 cells/mL. Samples from each concentration were taken and assayed using Guava ViaCount Flex on the Guava PCA system (blue diamonds) and trypan blue exclusion on a hemacytometer (pink squares). The average total cell concentration results from each assay are plotted against the expected cell concentration.

ments. The determinations for Total Cells/mL were linear over the test range, from about 10^4 to 5×10^5 cells/mL. The data show high correlation coefficients ($R^2 > 0.99$) and excellent correspondence for total cell counts from both methods (slope = 1.07 for trypan blue exclusion; slope = 0.98 for ViaCount Flex). The viability of the test culture samples did not change significantly over the dilution range during the experiment.

Instrument-to-instrument variability. Three (3) concentrations of Sf9 cells were assayed using ViaCount

Flex on three (3) different Guava PCA instruments. We designed the experiment to test cell counting at low, medium and high cell concentration: approximately 10^4 , 1.5×10^5 , and 5×10^5 cells/mL, respectively. Ten (10) replicate samples were prepared for each concentration, and data was acquired from each replicate sample twice, per instrument (N = 20 measurements). The data are summarized on Tables 1 and 2. Table 1 presents the average values for total cell concentration and % viability for each Sf9 cell sample run on the three (3) different instruments. The results agree closely between the different instruments. There is less than 3% difference in the cell concentration and % viability results among the three instruments tested. Table 2 shows the % CV values corresponding to the data collected for each culture from the three different Guava PCA instruments. The results obtained had excellent precision, with all %CV values <5% for total cell counts and %CV values <1.5% for viabilities.

Operator-to-operator variability. The variability seen from operator-to-operator was evaluated by three laboratory personnel running seven (7) Sf9 cultures that ranged in concentration from 1×10^4 to 5×10^5 cells/mL. Each operator prepared replicate samples from the cultures using ViaCount Flex and trypan blue for manual hemacytometer counting. A comparison of total cell count results from the three

	HIGH CONCENTRATION		MEDIUM CONCENTRATION		LOW CONCENTRATION	
Guava PCA Instrument	Cell Count (cells/mL)	% Viability	Cell Count (cells/mL)	% Viability	Cell Count (cells/mL)	% Viability
Unit 10	4.81×10^5	96.0	1.75×10^5	95.6	1.08×10^4	93.0
Unit 17	4.92×10^5	96.0	1.75×10^5	95.7	1.09×10^4	93.2
Unit 98	4.84×10^5	95.8	1.74×10^5	95.3	1.07×10^4	92.3

TABLE 1. Average cell counts and viabilities obtained from three (3) different Guava PCA instruments. Each table entry was calculated from N=20 data points.

	HIGH CONCENTRATION		MEDIUM CONCENTRATION		LOW CONCENTRATION	
Guava PCA Instrument	%CV of Cell Count	% CV of Viability	%CV of Cell Count	% CV of Viability	%CV of Cell Count	% CV of Viability
Unit 10	4.2	0.8	3.8	0.9	4.5	1.3
Unit 17	3.8	0.7	4.5	0.7	4.6	0.8
Unit 98	3.1	0.7	3.5	0.7	3.6	0.7

TABLE 2. Precision results (%CV) for cell counts and viabilities obtained from three (3) different Guava PCA instruments. Each %CV value was calculated from N=20 data points.

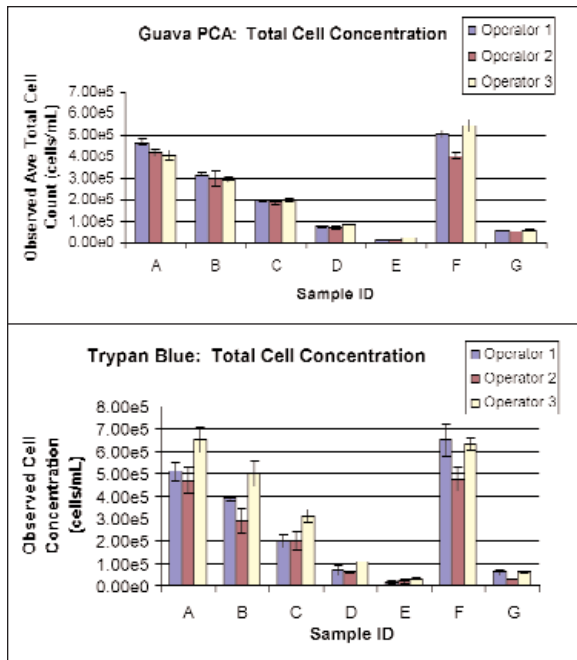


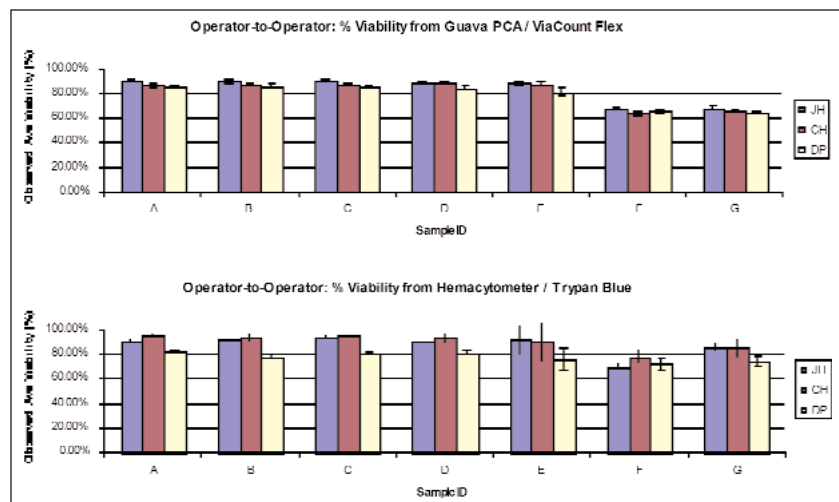
FIGURE 5: A) [TOP GRAPH] Comparison of total cell count results from three different operators for 7 different Sf9 cultures assayed using ViaCount Flex on the Guava PCA system. Each operator prepared replicate samples independently from the same stock culture and performed the ViaCount Flex assay. The same Guava PCA instrument was used by all three operators in this study. Average total cell concentrations (N=6) are plotted, with standard deviations indicated by the error bars. B) [BOTTOM GRAPH] Comparison of total cell count results from three different operators for the same seven (7) Sf9 cultures, above, stained with trypan blue and counted manually using a hemacytometer. Each operator prepared replicate samples independently from the same stock culture and performed manual counts at the microscope. Average total cell concentrations (N=2) are plotted, with standard deviations indicated by the error bars.

operators obtained using the Guava PCA system vs. trypan blue manual counts is shown in Figure 5. Figure 6 shows the comparison of % Viability results for the corresponding samples from the three operators using the Guava PCA system and trypan blue, hemacytometer assay. There was greater variability seen in both the total cell count and % viability results when performing manual trypan blue counts. The greatest discrepancy in counts between the 3 operators was calculated as a % difference and tabulated in Table 3. The % difference in results was consistently greater for trypan blue manual counts than the ViaCount Flex assay on the Guava PCA system.

TABLE 3. The maximum % difference in results obtained from three independent operators assaying the same culture samples with the Guava PCA system and hemacytometer counts.

Culture	% DIFFERENCE IN TOTAL CELL COUNT		% DIFFERENCE IN VIABILITY	
	Guava PCA & ViaCount Flex	Hemacytometer & Trypan Blue	Guava PCA & ViaCount Flex	Hemacytometer & Trypan Blue
A	13	38	5	14
B	8	72	4	17
C	5	55	5	15
D	23	81	6	12
E	82	118	8	17
F	35	33	4	11
G	11	105	5	14

FIGURE 6: A) [TOP GRAPH] Comparison of % Viability results from three different operators for 7 different Sf9 cultures assayed using ViaCount Flex on the Guava PCA system. Each operator prepared replicate samples independently from the same stock culture and performed the ViaCount Flex assay. The same Guava PCA instrument was used by all three operators in this study. Average % Viability results (N=6) are plotted, with standard deviations indicated by the error bars. B) [BOTTOM GRAPH] Comparison of % Viability results from three different operators for the same seven (7) Sf9 cultures, above, stained with trypan blue and counted manually using a hemacytometer. Each operator prepared duplicate samples independently from the same stock culture and performed manual counts at the microscope. Average total cell concentrations (N=2) are plotted, with standard deviations indicated by the error bars.



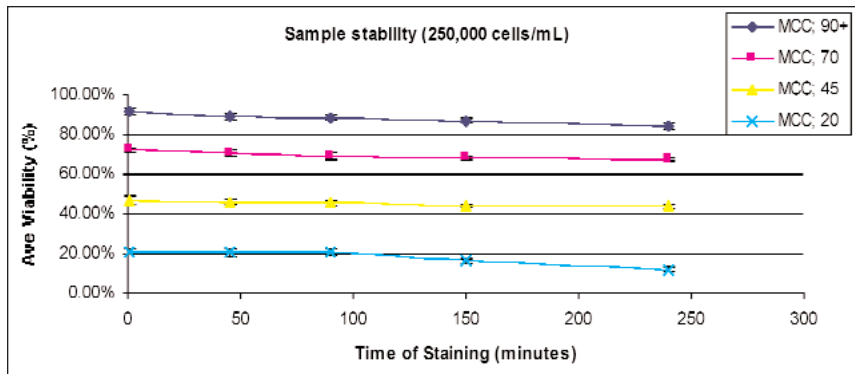


FIGURE 7: Time course study from a representative post-stain stability assessment. The results from four (4) test samples at viabilities of about 20% (cyan “X”), 45% (yellow triangle), 70% (pink square) and 90+% (blue diamond) are shown here. All samples were at the cell concentration of 2.5×10^5 cells/mL.

Assay results were more reproducible for operators using the Guava PCA system. The % CV for replicate total cell counts from three (3) different operators ranged from 5% – 14% for the Guava ViaCount assay vs. 16% - 41% for hemacytometer counting. The % CV for replicate viability measurements of Sf9 cultures from three (3) different operators was 2% - 4% for the Guava ViaCount assay vs. 6% - 14% for hemacytometer counting of trypan blue-stained cells.

Post-stain sample stability. Twelve (12) Sf9 stocks were prepared from four (4) cultures at different viabilities: about 20%, 50%, 70% and 90+%. Each stock was diluted to yield three different cell concentrations (5×10^5 cells/mL, 2.5×10^5 cells/mL, and 5.0×10^4 cells/mL). Replicate samples were prepared from each of the twelve (12) Sf9 stocks. Over a planned time course, samples were stained with ViaCount Flex. Each stained sample was acquired on the Guava PCA system after the 5-minute incubation period, then at 45, 90, 150, and 240 minutes to observe the post-stain stability of the sample. Samples were held at room temperature, shielded from light exposure during the time course experiment. The stability of the cell sample was monitored by plotting the viability over time. Figure 7 shows representative post-stain stability assays for four (4) test samples at the mid-range cell concentration. The mean fluorescence intensities (PM1 and PM2) for viable cells and non-viable cells showed little change over the 4 hour time course. Table 4 summarizes the stability of the cell concentrations at different viabilities. The sample was deemed “stable” if there was less than a 5% decrease in viability from the first measurement, 5 minutes after staining. For example, the low 20% viability sample at high cell concentration was stable for

45 minutes; after 45 minutes, the viability decreased by more than 5% from the starting value. For the higher viability samples at high cell concentration, the samples maintained their viability level to within 5% of the starting value for at least 150 minutes from staining. Sf9 cells maintain their viability longer when using ViaCount Flex, allowing users more flexibility in the time between staining cells and acquiring data on the Guava PCA system.

TABLE 4. ViaCount Flex post-stain sample stability of Sf9 cultures at different cell concentrations and viabilities.

Sample	TIME VALUE (IN MINUTES)			
	90+ viability	70% viability	50% viability	20% viability
HCC	150	240+	150	45
MCC	90	90	90	90
LCC	90	150	150	45

HCC = High cell concentration (5×10^5 cells/mL)
MCC = Mid cell concentration (2.5×10^5 cells/mL)
LCC = Low cell concentration (5.0×10^4 cells/mL)

Conclusion

The Guava ViaCount Flex assay expands the utility of the Guava ViaCount application to non-mammalian cell lines that can be problematic for the standard ViaCount reagent. The assay was optimized and validated using Sf9 insect cells that were adapted to suspension culture in serum-free medium. The Guava ViaCount Flex yielded results that showed good correspondence and linear correlation to trypan blue hemacytometer counts ($R^2 > 0.99$) over a

broad range of cell densities (10^4 to 5×10^5 cells/mL) and culture conditions (75 - 90%). Assay results were highly reproducible, with % CV for replicate cell count values, ranging from 0.95 - 6.3%, and % CV for replicate viability measurements of 0.89 - 3.46% (single instrument, single user). Significantly better operator-to-operator reproducibility is attained using ViaCount and the Guava PCA system vs. manual counting with trypan blue exclusion. The assay is easy to perform, with rapid data acquisition and analysis on the Guava PCA system. The Guava Via-count Flex reagent expands the utility of the ViaCount application to provide realistic assessments of insect and other non-mammalian cell lines, allowing better control over cells used in experimental processes.

References

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