

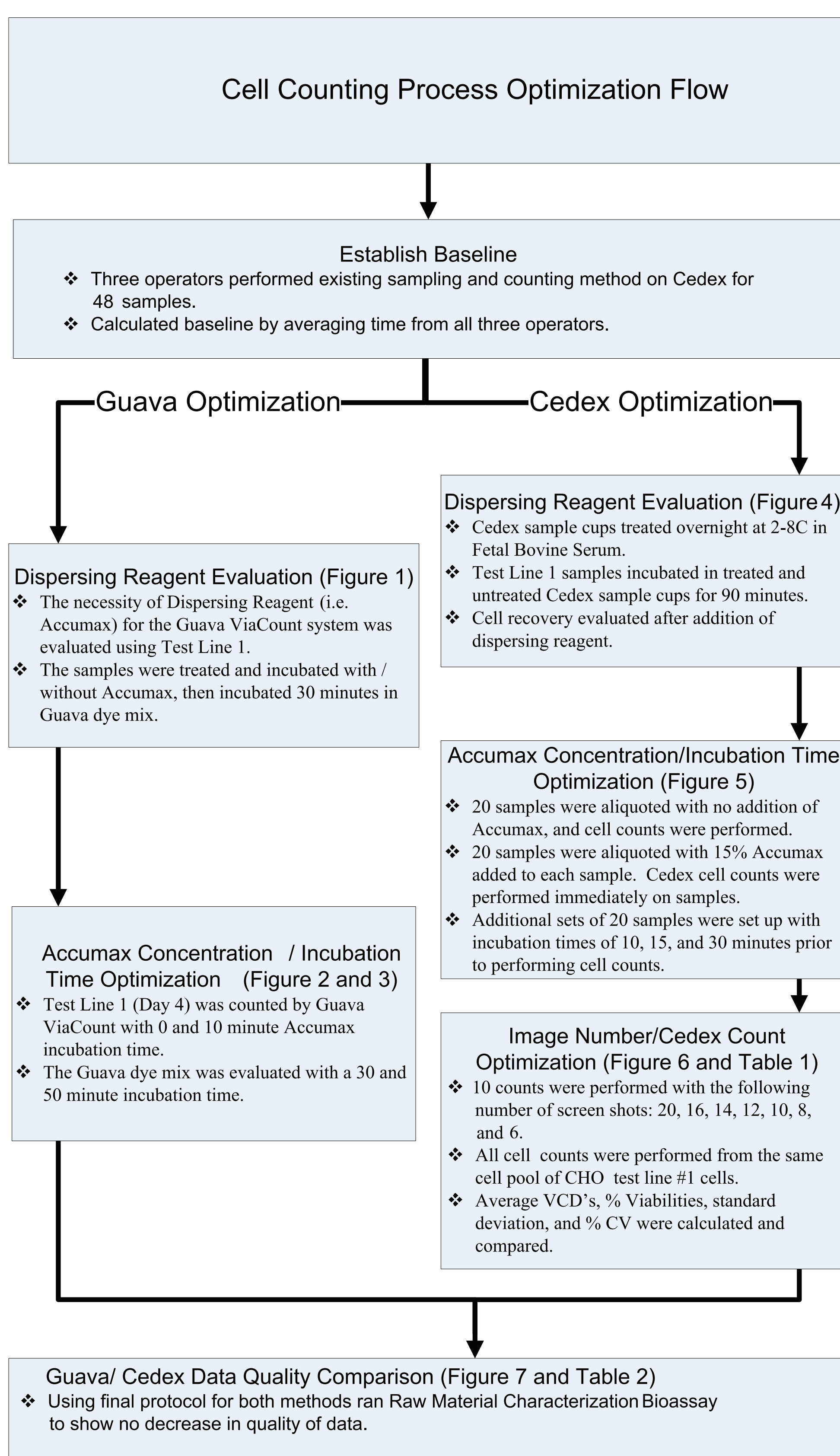
Abstract

The development of cell culture media and processes typically requires screening large numbers of cultures or conditions. Monitoring cell density is a key parameter for evaluating culture performance. The development of automated viable cell counters based on the trypan blue exclusion method has significantly increased throughput, improved consistency and reduced the labor in this process. However, these cell counters generally have relatively limited sample queues (up to 20 samples) and long processing times (>3 minutes per sample). SAFC's raw material characterization (RMC) program, for example, requires analysis of more than 200 samples per day, exceeding 11 hours of instrument analysis time. In order to improve cell counting throughput, methods to reduce sample analysis time with the Cedex cell counter and alternate counting methods were investigated.

The Cedex cell counting process was evaluated with three test cell lines used for the RMC program, including two CHO cell lines. Multiple steps of the process were considered to reduce the cell counting cycle time. The process was also optimized to address an issue of cell adhesion to plastic surfaces. Modifications in the process were compared to original method to ensure there was no loss in accuracy or precision. The results for the Cedex process optimization show that the cycle time per sample could be reduced by >20% without negatively impacting the reliability of the method.

In order to find alternative methods to the Cedex cell counter and to achieve higher throughput, Guava cell counting technology was investigated. The Guava ViaCount system is capable of counting samples in a 96 well plate format. The Guava process was optimized to achieve counts comparable to the reference Cedex counting method using CHO-K1 (Test Line 1) as a model cell line. A cell dispersing reagent, optimization of incubation time and the sample handling were identified as key variables affecting cell counts with this method. The Guava method was refined to achieve a 2-fold reduction in cell counting process time.

Method Flow



Guava Optimization

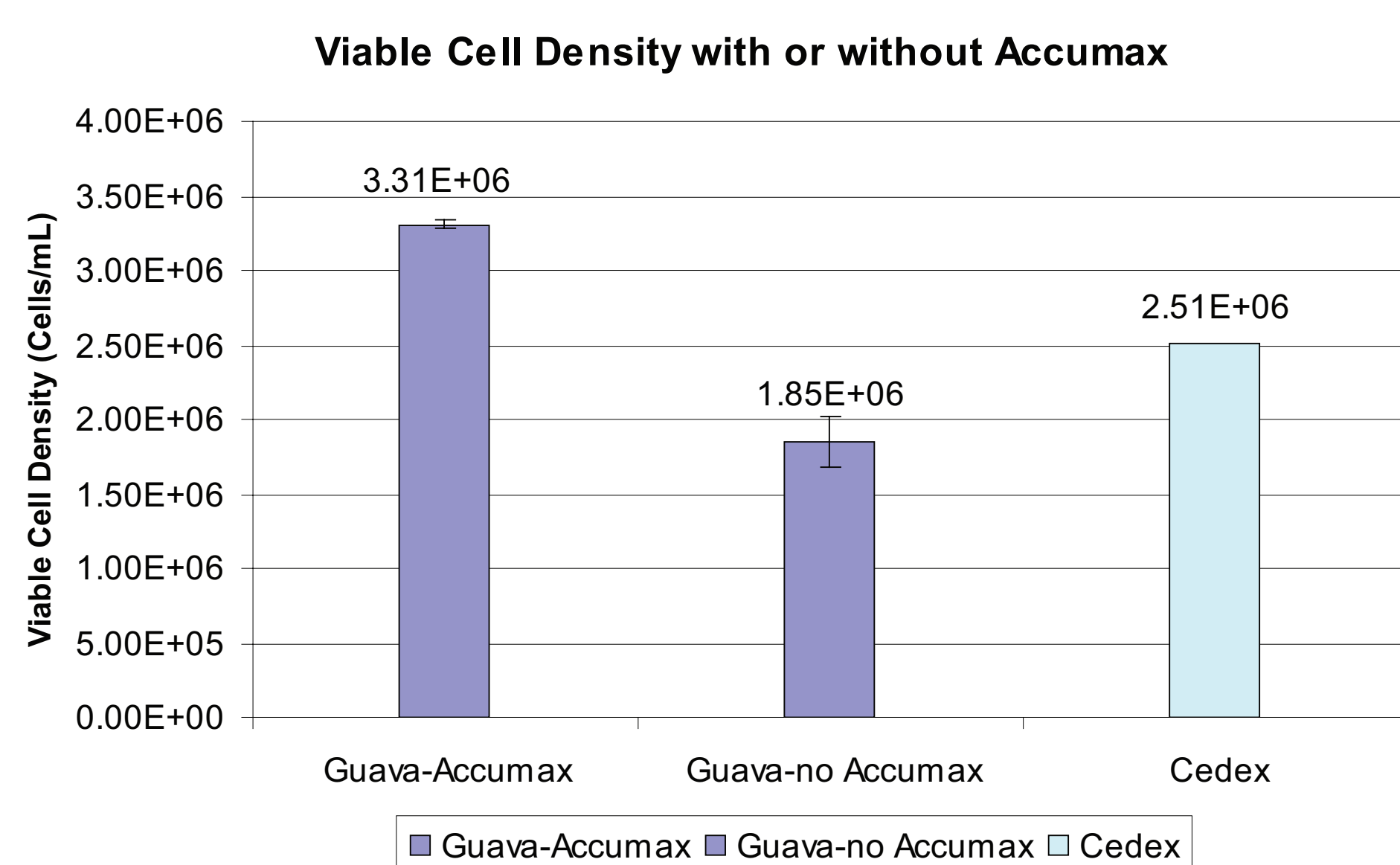


Figure 1

The requirement for dispersing reagent (Accumax) addition was evaluated. The Day 4 Test Line 1 culture samples were incubated at 37 °C for 15 minutes with or without 25% Accumax (final concentration). Dye mix is added to the sample/Accumax mixture and incubated 30 minutes in a 96-well plate. Samples read by Guava ViaCount to determine viable cell density and % viability. The Cedex count was added to the graph as a comparison.

Results:

The condition of 15 minutes incubation with Accumax presented higher VCD, and smaller standard deviation than one without incubation.

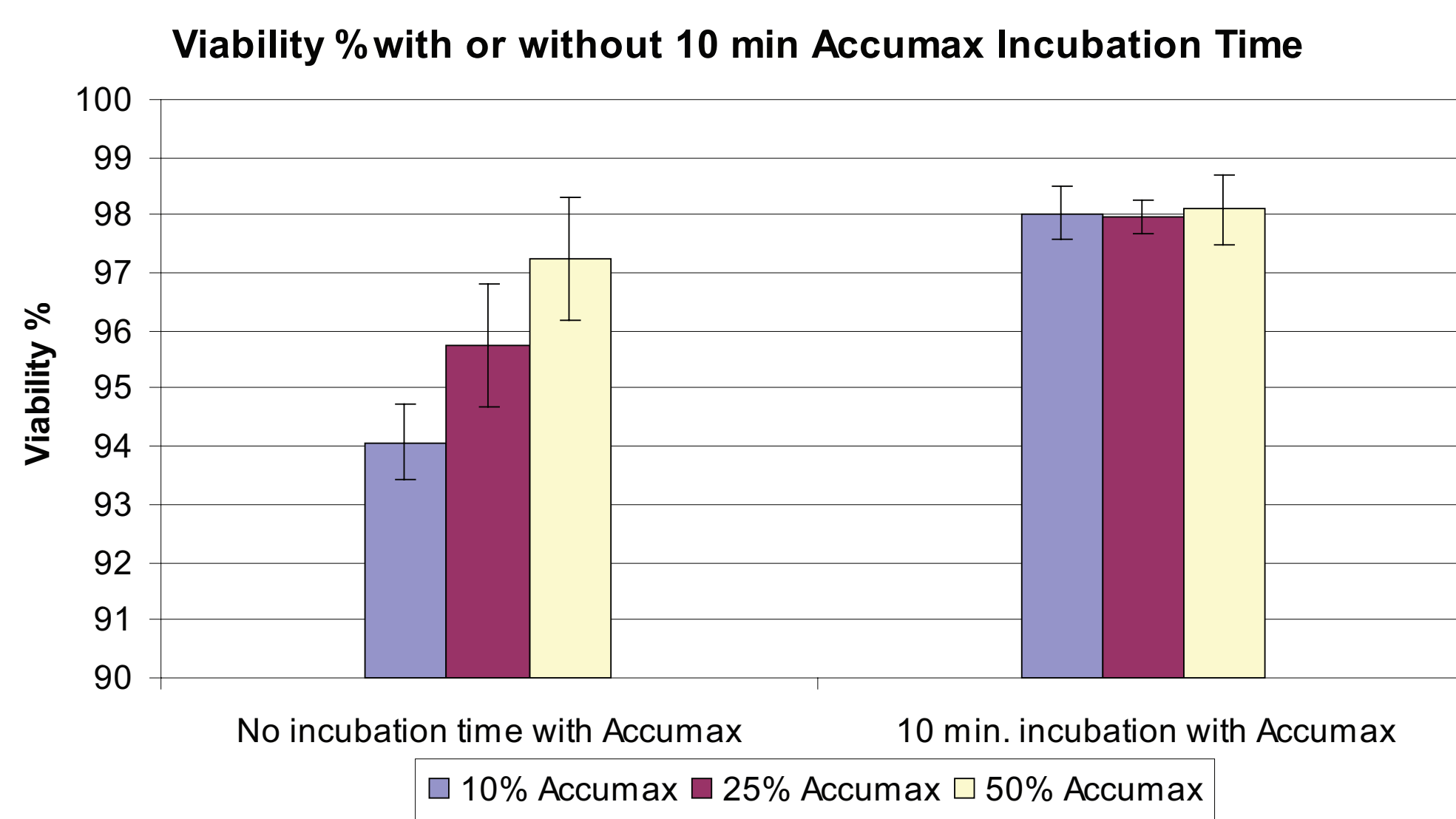


Figure 2

Viability percentage among the experimental groups is shown with no Accumax incubation and a range of Accumax concentrations. All experimental conditions were incubated with Accumax followed by Guava Dye mix for 30 minutes before the cell count.

Results:

Ten minutes with Accumax incubation followed by 30 minutes with Guava Dye mix incubation increased the viability % compared to the groups without Accumax incubation time. However, the percentage of Accumax concentration did not affect the cell culture viability % when the samples were incubated for 10 minutes with Accumax.

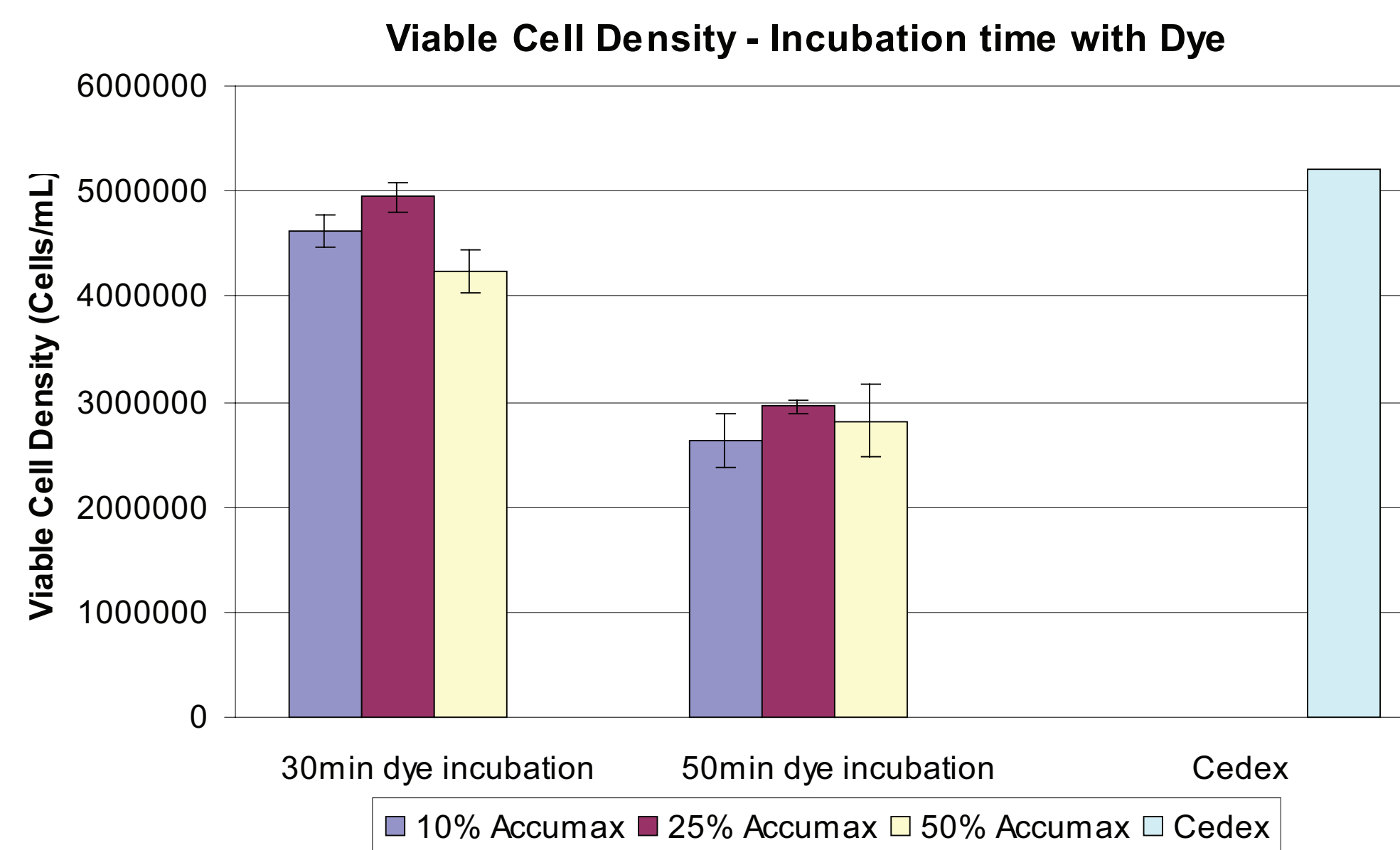


Figure 3

Guava dye mix incubation time was evaluated in order to achieve comparable counts to the Cedex.

Results:

Thirty minutes incubation at 37 °C with Guava dye resulted in counts that were closer to the Cedex control and smaller standard deviations than the 50 minutes dye incubation group. However, the % viability of 50 minutes incubation with Guava Dye mix did not show significant difference from that of 30 minutes incubation (data not shown).

There was no significant difference among three Accumax concentrations in the viable cell density.

Cedex Optimization

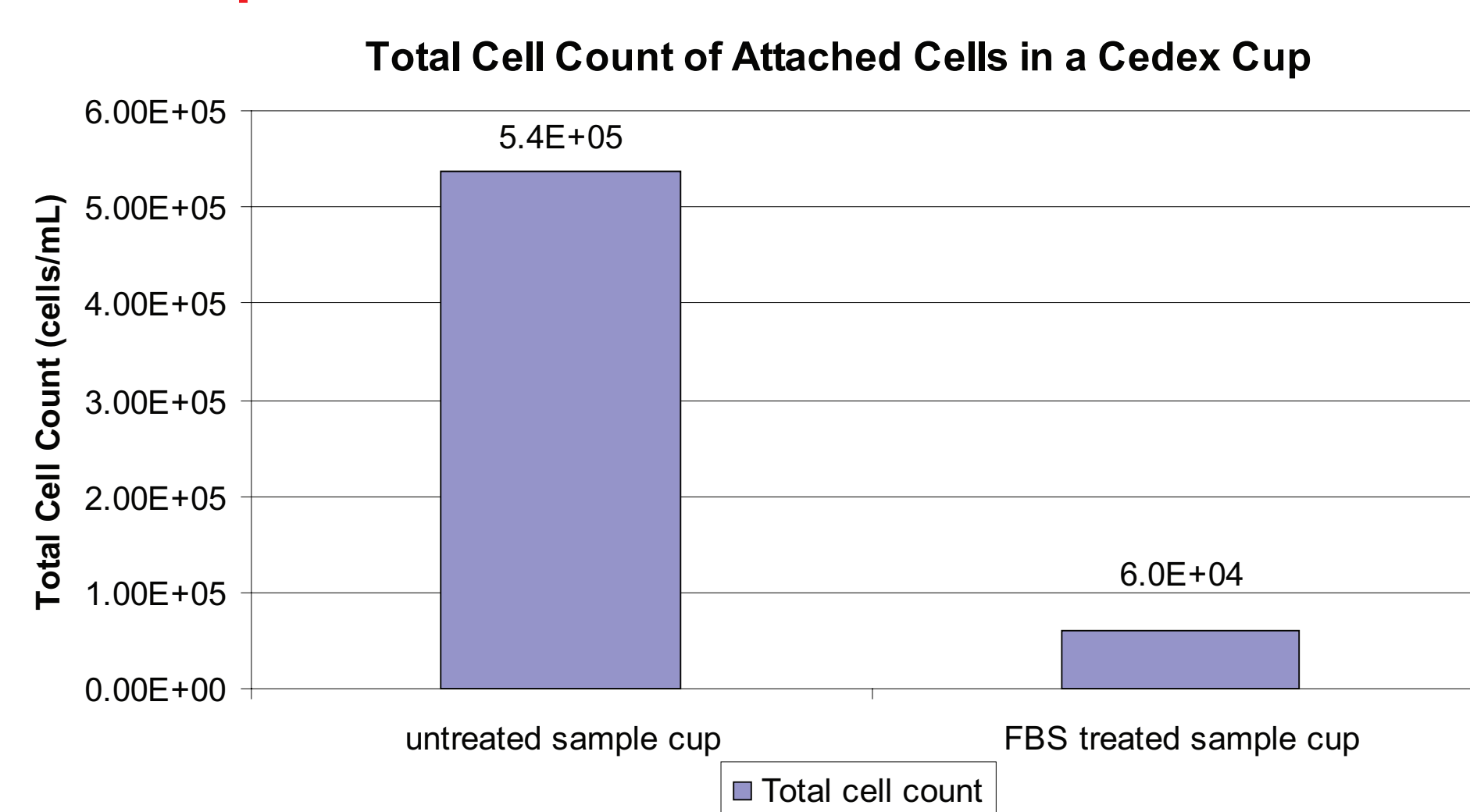


Figure 4

Test Line 1 samples were added to untreated Cedex sampling cups and sampling cups treated with FBS at 2-8°C overnight, then incubated at room temperature for 90 minutes. All cups were then emptied, and 50% Accumax was added to detach any possible cells attached to the plastic sample cups. Accumax was incubated in the sampling cups for 10 minutes before the sample was counted with the Cedex.

Results:

6.0 x 10⁴ cells/mL were recovered from the FBS treated sample cups, while 5.4 x 10⁵ cells/mL were recovered from the untreated Cedex sample cups. The almost ten fold increase in cells recovered from the untreated sample cups verified that Test Line 1 was adhering over time to the plastic cups, thus significantly impacting the viable cell count data.

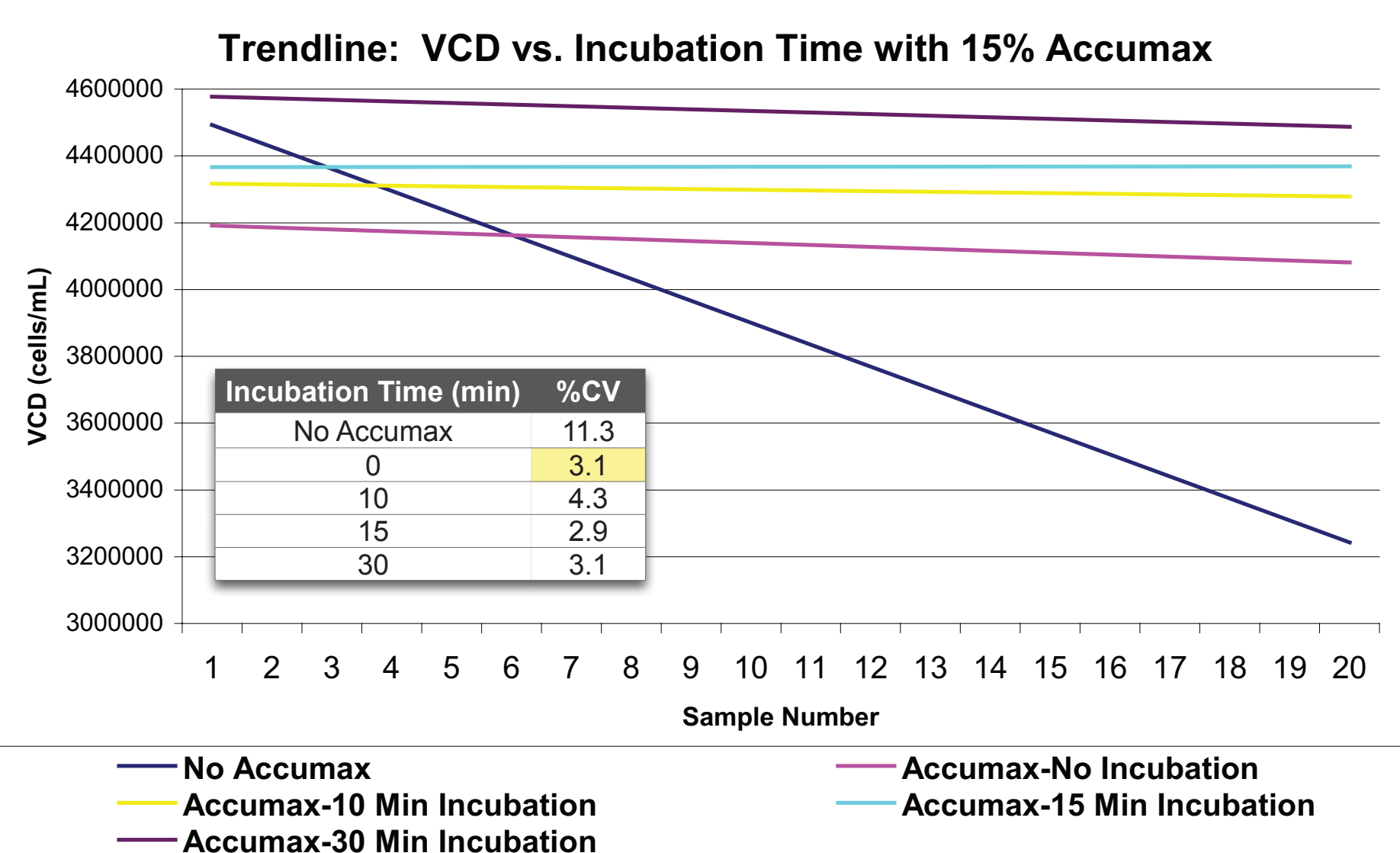


Figure 5

Test Line 1 incubated with and without Accumax (15%) for varying times. The 15% Accumax concentration was selected as the optimal concentration based on earlier experiment (data not shown). 20 samples were aliquoted with no addition of Accumax, and cell counts were performed. After completion of the initial 20 samples, an additional Twenty samples were aliquoted with 15% Accumax added to each sample. Cedex cell counts were performed immediately on samples. Additional sets of 20 samples were set up with Accumax incubation times of 10, 15, and 30 minutes prior to performing cell counts.

Results:

Reduction of % CV and standard deviation for VCD was observed in conditions where 15% and 45% (data not shown) of Accumax were added to the sample.

No significant difference in % viability was observed for any condition. The 15% addition of Accumax was selected to continue optimization as it minimized the addition of reagent to sample but still decreased standard deviation and % CV in relation to the untreated sample (see inset table).

Figure 5 shows that a significant decrease in VCD was observed in the samples with no addition of Accumax as the 20 samples were counted. With the addition of Accumax, the reduction in VCD was not observed. In addition, a significant improvement in standard deviation and % CV was observed (see inset table). No improvement was realized by adding an incubation step prior to initiating the cell counting process immediately following the addition of the Accumax. Therefore, the Cedex cell counting process would not include any incubation step following the addition of the Accumax.

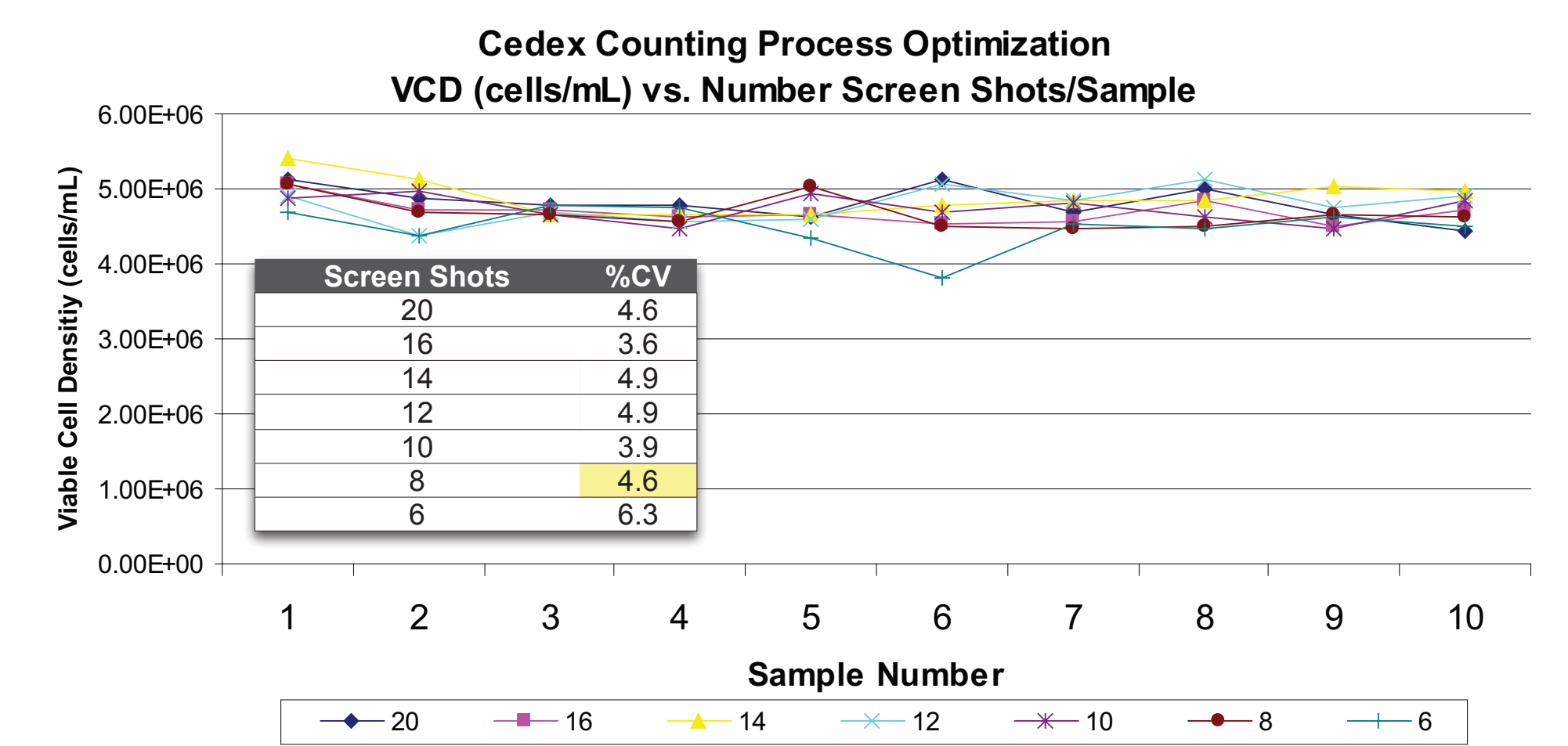


Figure 6

The default value of screen shots that is counted for each sample on the Cedex is 16 screen shots/sample. To evaluate the impact of reducing the number of screen shots on data quality, 10 counts were performed on Test Line 1 day 5 post-seeding with the following number of screen shots: 20, 16, 14, 12, 10, 8, and 6. All cell counts were performed from the same cell pool of Test Line 1.

Results:

No significant changes in standard deviation or % CV were observed by reducing the number of screen shots from 20 per sample to 8 per sample (see figure 6). Additional dates post-seeding were tested (not shown) and exhibited similar results. Modification of the cell counting protocol to eight screen shots per sample was initiated as there was no negative impact observed on data quality (see inset table), and it would result in a significant reduction in analysis time per sample.

Screen Shots	Time (20 Samples)	Time Savings (%)
20	75.9	-10.0
16	66.8	0.0
12	59.9	10.0
10	57.2	14.0
8	53.4	20.0
5	48.8	27.0

Table 1

Reduction of the Cedex screen shots per sample has resulted in a significant decrease of analysis time. The 20% decrease in analysis time has been achieved by modifying the process to count eight screen shots per sample without decreasing the quality of data (optimized condition highlighted in yellow).

Summary

Correlation between Guava vs. Cedex VCD counts D0-D7

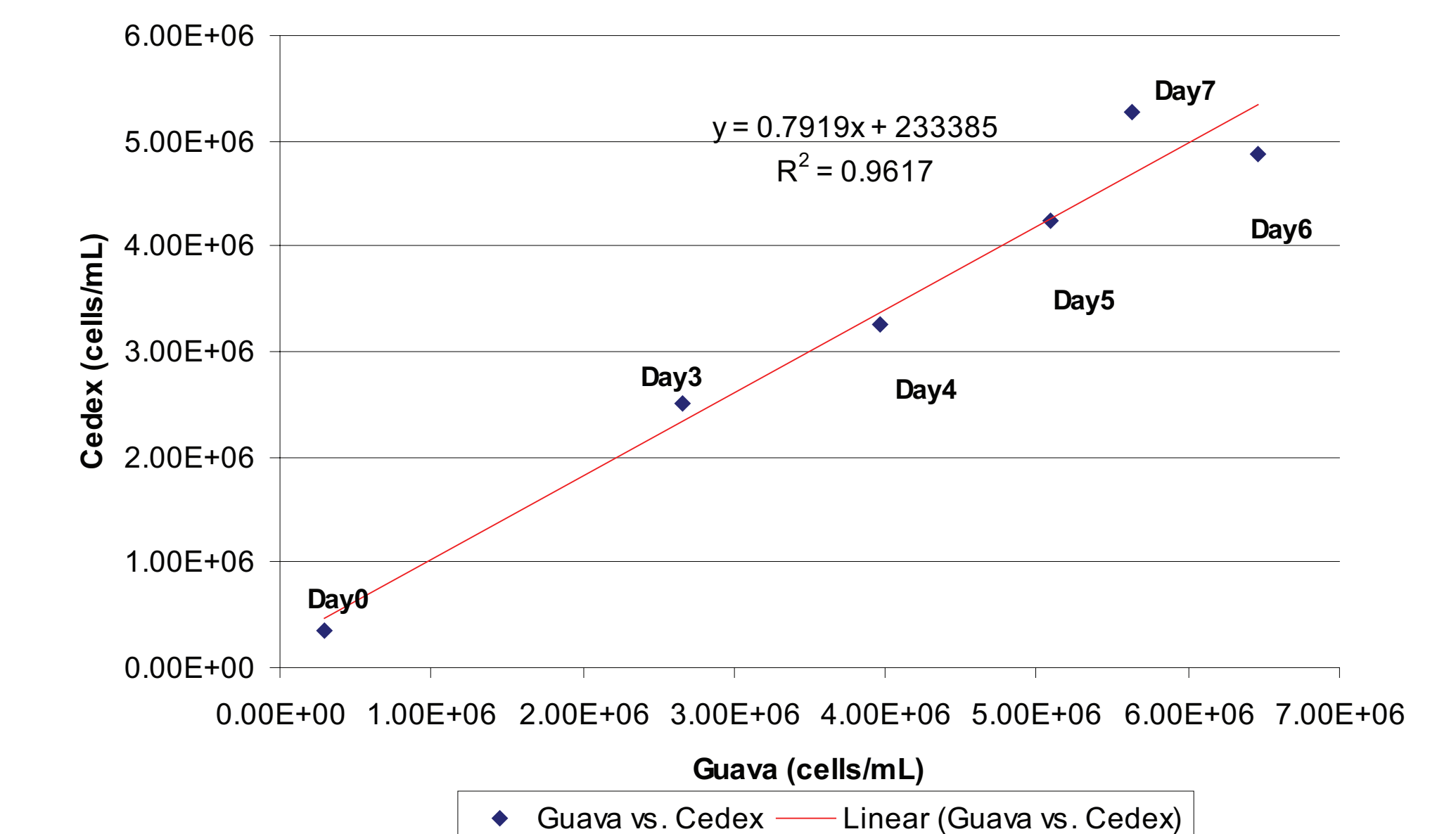


Figure 7

The correlation between Guava ViaCount and Cedex optimized process was evaluated using Test Line 1. The daily counts of 48 individual samples from Day 0 to Day 7 were performed using both Guava ViaCount and Cedex. The conditions for Guava ViaCount were set to 25% Accumax addition with 10 minutes incubation, and 30 minutes incubation in the Guava dye mix. The data were plotted in correlation regression curve.

Results:

The correlation regression with $R^2 = 0.96$ indicated there was good correlation between Guava and Cedex count. The slope=0.79 suggested that the cell counts by Guava ViaCount were slightly higher than by Cedex. Removing the Day 7 count increased the correlation to $R^2 = 0.98$. The reduced correlation on Day 7 could be because of an increase in measured dead cell count in Guava ViaCount system.

Summary Table

	Cedex Baseline	Cedex Optimized	Guava
Accumax Addition (%)	N/A	15	25
Accumax Incubation (min)	N/A	0	10
Time/48 Samples (min)	208	166	86
Replicate %CV	11.3	4.6	6.2
% Time Reduction	N/A	20	59

Table 2

Summary of optimized Cedex and Guava cell counting process. Both have resulted in a significant reduction of analysis time, as well as improving the % CV when compared to the Cedex baseline method.

Conclusion

- The optimized Cedex cell counting process has resulted in a 20% reduction in the analysis time. No negative impact on data quality has been observed for all three cell lines that are utilized in the Raw Material Characterization program.
- The Cedex % CV was improved by the addition of Accumax for Test Line 1, thus increasing the quality of the data generated while decreasing the analysis time. After testing on multiple cell lines the requirement for addition of Accumax proved to be cell line specific.
- The optimized Guava 96PCA ViaCount process resulted in a time reduction of 59% when compared to the Cedex baseline data with a similar % CV compared to the optimized Cedex method.
- Both optimized processes are improved counting methods for the Raw Material Characterization program. Guava 96PCA ViaCount could be used for a high throughput method alternative for Cedex.

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