Evaluation of the Mixing Effect of the CytScop® Pro

Introduction

As we all know, the CytScop® Pro is designed with a rotating sample tray that can test 24 samples at a time, taking approximately 1 hour to test all 24 samples. During the testing process, samples gradually settle, and if the samples are not thoroughly mixed before sampling, the accuracy and precision of the measurement results cannot be guaranteed. The CytScop® Pro is equipped with an automatic mixing device before sampling to ensure that the samples to be tested, which have been sitting for a long time, are thoroughly mixed before sampling to ensure the uniformity of sample concentration before sampling. However, it is necessary to further verify and explore whether re-mixing samples that have been sitting for a long time will affect the samples, leading to deviations in viability, diameter, or concentration from the true values.

To determine whether mixing the samples before sampling with the instrument after a long period of sitting affects the samples, we used cell samples with a certain viability and concentration. Samples from the same source were tested using multiple CytScop® Pro devices with a resting time of 0-2 hours. We analyzed the mixing effect of the instruments and their impact on the samples.

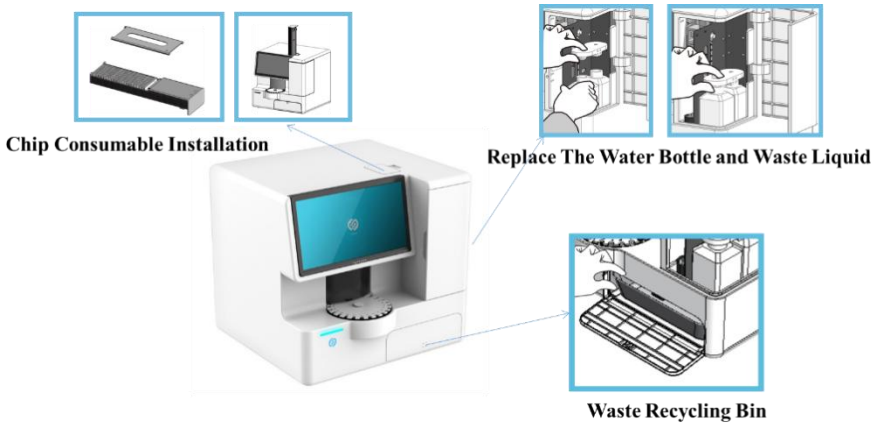

Figure 1. CytScop® Pro

Methods

The prepared samples, a total of 60 samples, were taken out all at once. Starting the timer from the extraction of the last sample, the samples were allowed to sit for 0h, 0.5h, 1h, 1.5h, and 2h respectively. Four CytScop® Pro devices were randomly selected for machine testing, with 3 parallel samples randomly taken from the extracted samples for each device and each resting time group to ensure the randomness of sampling and reduce sampling errors. Additionally, for the samples left to sit for 0 hours, manual mixing was performed before machine testing.

Resting time/h	Instrument 1	Instrument 2	Instrument 3	Instrument 4

0	3	3	3	3
0.5	3	3	3	3
1	3	3	3	3
1.5	3	3	3	3
2	3	3	3	3
All samples	60			

Samples and instrument settings

Cell Sample

CHO cells, short for Chinese Hamster Ovary cells, are a commonly used mammalian cell line in biotechnology and biopharmaceutical research. Percentage viability and total Jurkat cell concentration are 90% and 7.5×10^6 cells /mL.

Test Mode

TB Stain

Instrument Settings

Cell type	CHO
Number of sampling areas	3
Settling time (s)	30
Mixing frequency	3
Minimum cell diameter	8
Maximum cell diameter	24
Agglomeration factor	0.8
Live cell brightness	2
Dead cell brightness	10
Dead cell coefficient	2
BF (mu s)	18000
gain	0

Results

Randomness in sampling can effectively reduce non-systematic errors in the entire measurement process. The coefficient of variation (C.V) is a statistical measure used to assess the variability of each observed value, and in instrument measurements, it can reflect the precision of the instrument's measurements. As shown in Figures 2 and 3 below, the C.V values for total cell concentration (TCD) and viable cell concentration (VCD) measured by 4 CytScop® Pro devices are presented.

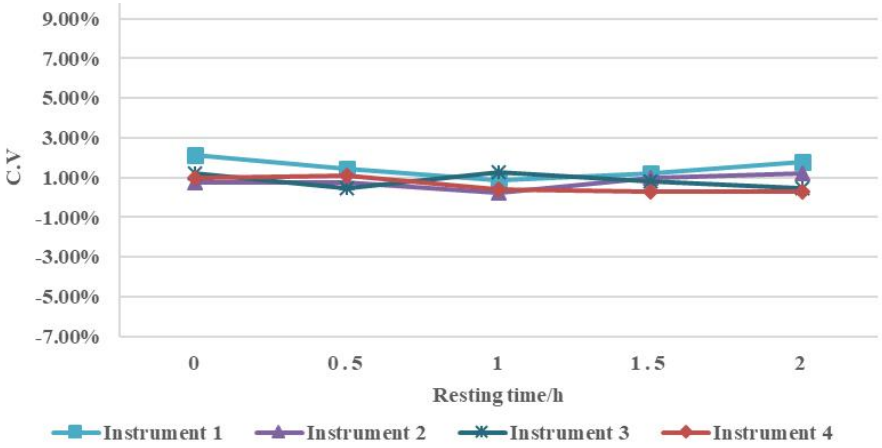

Figure 2. The C.V values of measuring sample TCD at different settling times.

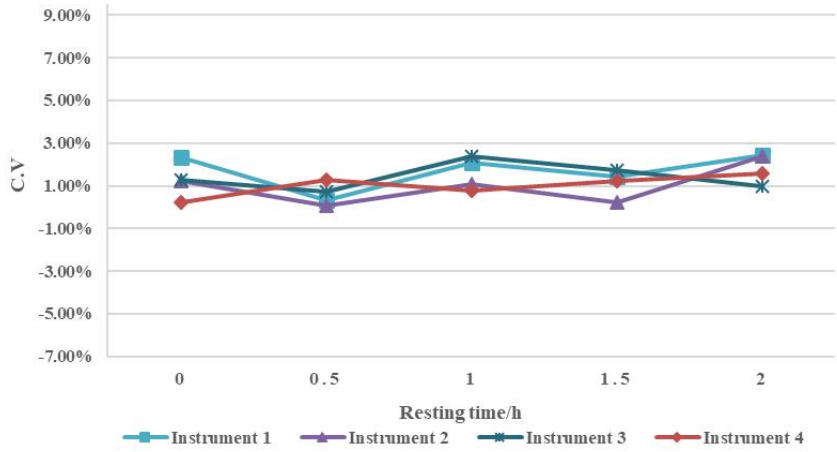

Figure 3. The C.V values of measuring sample VCD at different settling times.

Statistical analysis of measuring TCD and VCD by 4 instruments at different settling times, as shown in Figures 4 and 5.

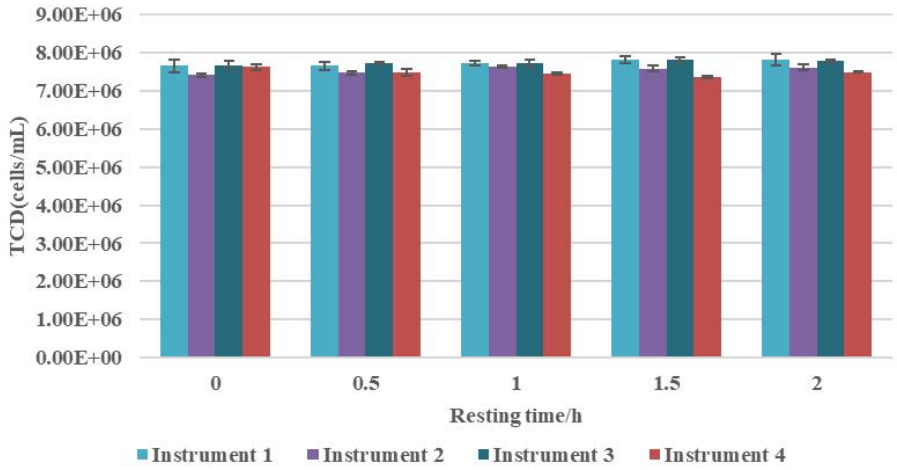

Figure 4. The variation in TCD measurements by 4 instruments at different settling times.

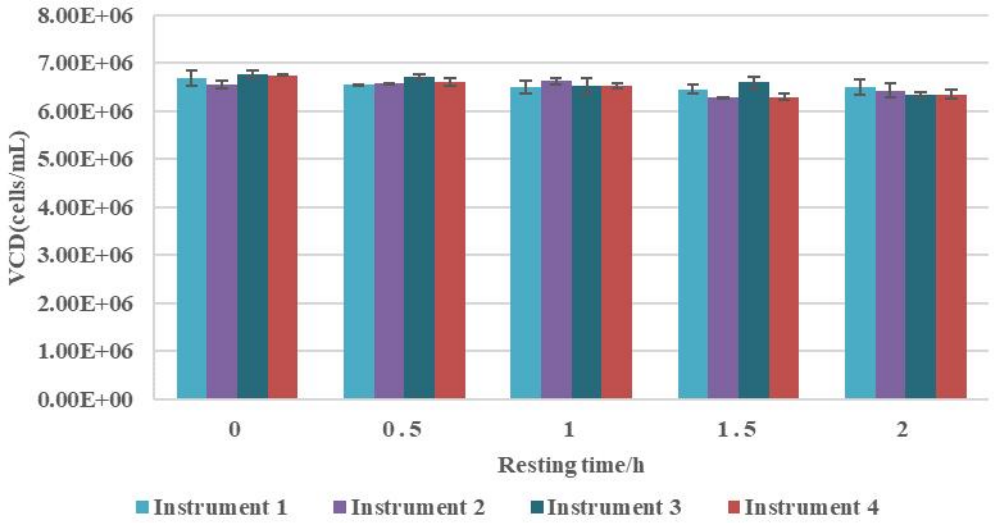

Figure 5. The variation in VCD measurements by 4 instruments at different settling times.

The variation in sample diameter (Dia) and viability (Via) measurements by 4 instruments at different settling times, as shown in Figures 6 and 7. Statistical analysis of the changes in TCD, VCD, and Via measurements from 0h to 2h is presented in Table 1.

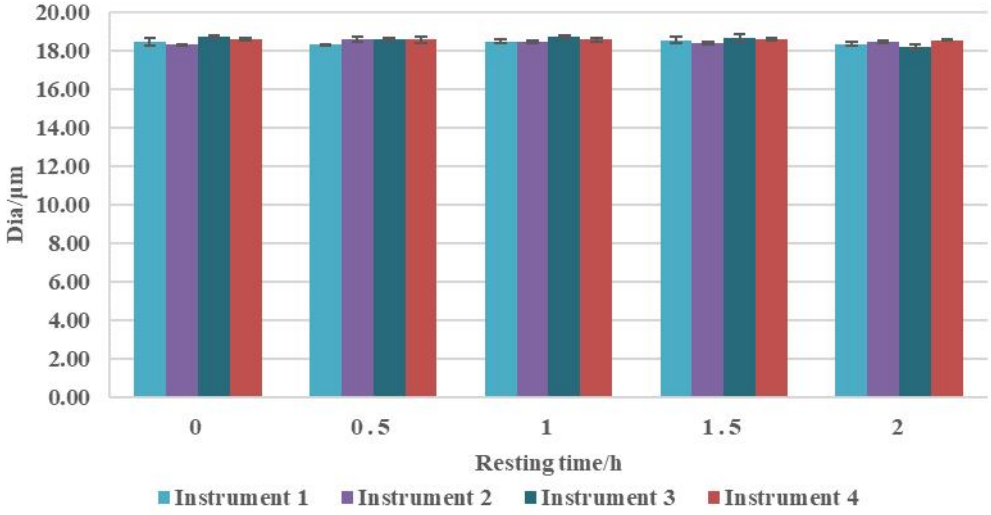

Figure 6. The variation in Dia measurements by 4 instruments at different settling times.

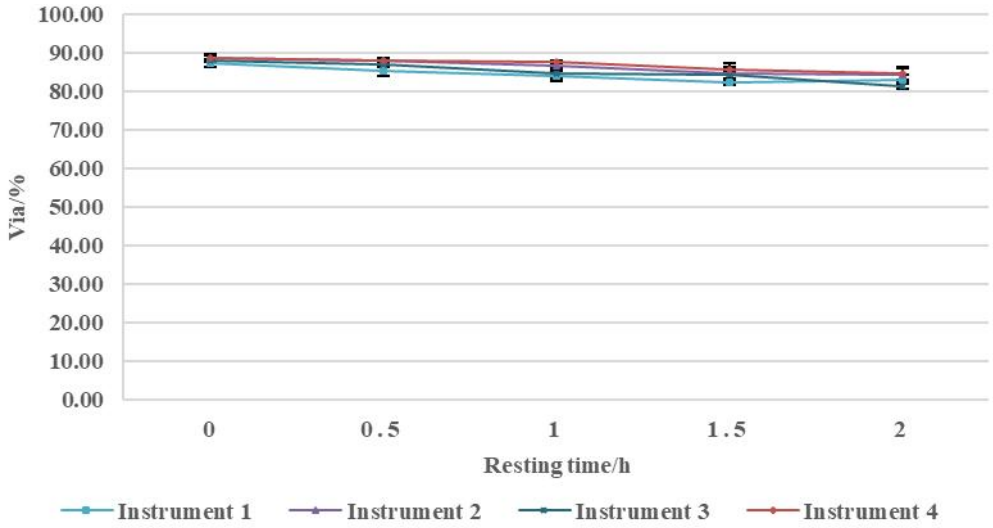

Figure 7. The variation in Via measurements by 4 instruments at different settling times.

Table 1 Difference of sample measurement results at 0 hours and 2 hours standing time

CytScop Pro	TCD/%	VCD/%	Via/%
Instrument 1	**+ 2.09**	**2.69**	**4.72**
Instrument 2	**+ 2.83**	**1.98**	**4.74**
Instrument 3	**+ 1.43**	**2.98**	**4.81**
Instrument 4	**1.7**	**2.86**	**4.39**
Note: Percent difference = (2h measured value -0h measured value) /0h measured value; "-" means that the measurement is lower compared to 0h, and "+" means that the measurement is higher compared to 0h			

Conclusion

Four randomly selected CytScop® Pro instruments demonstrated excellent measurement repeatability by conducting on-machine measurements of samples settled for 0h-2h, with Coefficient of Variation (C.V) analysis for TCD and VCD (as shown in Figure 2 and Figure 3). All four instruments exhibited very good measurement repeatability, with C.V values all within 3%, indicating high accuracy and low systematic errors of the instruments.

Compared to measurements of samples settled for 0h, measurements of TCD and VCD for samples settled for 2h on the same instrument showed no significant differences ($p > 0.05$). The concentration changes after settling for 2h compared to 0h were less than 3% (as shown in Table 1). Different instruments (as shown in Figure

4 and Figure 5) also did not exhibit significant differences in measuring sample concentrations at the same settling time, indicating minimal inter-instrument variation.

Statistical analysis of diameter measurements, as shown in Figure 6, reveals that for the same instrument, sample diameter changes very minimally at different settling times, not exceeding 3%, with no significant differences ($p > 0.05$). Additionally, there were no significant differences in the test results of samples at the same settling time across different instruments. Comparative analysis of Via test results for the 4 instruments at various settling times (as shown in Figure 7) indicates a slight decrease in Via as the sample settling time increases. Compared to samples settled for 0h, Via decreased by an average of around 4.5% after settling for 2h. The decrease in viability is related to the duration of room temperature storage; the longer the storage time, the greater the viability decrease. Furthermore, the initial viability of the samples is relatively low (around 90%), indicating a moderate cell state, leading to a significant decrease in sample viability in measurements on the same instrument.

In summary, the CytScop® Pro intelligent cell analyzer demonstrates good mixing effects in measuring samples settled for 0h-2h. There were no significant differences in cell concentration measurements over time gradients, and the impact on cell viability was minimal, showcasing the CytScop® Pro's excellent stability in measuring a large number of samples.

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