High Throughput ZetaView® System vs High Cost of Other Methods

Introduction

In addition to using Nano Particle Tracking Analysis (NTA) to measurement the size distribution and concentration of EV samples, both Microfluidic Resistive Pulse Sensing (MRPS) and the Single Particle Interference Reflectance Image Sensor (SP-IRIS) methods have been widely used as an alternative means of characterizing EVs. In this note, we relay two specific cases for using the ZetaView® NTA system to achieve relative "high-throughput" analysis of many EV samples, along estimations of throughput for MRPS & SP-IRIS methods for an equivalent number of samples; further, we establish realistic estimates for the high cost of ownership for operating MRPS & SP-IRIS systems as a result of the cost of consumables as well as the substantially greater amount of time spent to run the same number of samples.

Case 1: ZetaView® Customer in North Carolina

Table 1 shows our calculation of how it is possible to complete 32 EV samples in 1 hour. In addition, we report the time required to run 10x single sample analyses (not triplicate), shown in Table 2. There is a reasonable agreement between the estimated time for running 32 samples and the time needed to run 10 standard samples (i.e., on a per sample basis). Further, although acquiring only single analyses would appear to avoid the requirement of collecting data in triplicate, our method produces results that are statistically overdetermined, given that we automatically measure 11 unique sub-volumes per sample, while in the cell.of samples.

Case 1: Estimated time to run 32 samples: Is this realistic in 1 hour?

2 seconds per NTA movie x 11 movies = 22 seconds of data acquisition
+ 18 additional seconds of instrument settling time during NTA capture
+ 30 seconds for NTA software data tracking
+ 10 seconds to produce a PDF data report and return to data acquisition = 22 + 28 + 20 + 10 = **80 seconds per single analysis**

Other Factors:
- samples are pre-diluted, and this assumes use of a single SOP
- the next sample put into a syringe while the previous sample is analyzed add 15 seconds of handling time plus settling time after sample injection

Total Time per Single Sample = 95 seconds

Minimum Analysis Time = 85 seconds/sample x 32 samples
for 32x samples = 95 x 32 = 3040 seconds = 50.7 minutes

Table 1: ZetaView® Throughput: Case 1, run 32 samples in 1 hour

100nm PSL beads were prepared for NTA analysis, as a validation of estimated productivity for Cases 1 & 2

Case 1: 10x single samples were run in series (not in triplicate)
- the same SOP and dilution factor (250,000x) were used
- movie length = 2 seconds @ 11 movies colledted/sample
- a different syringe was used for each analysis
- a rinse of the sample cell was done between each analysis

Total Time Elapsed to completion = 17 minutes, 21 seconds

= 1041 seconds for 10 samples = 105 seconds/sample for 32x samples = 105 x 32 = 3360 seconds = **56 minutes**

Case 2: 10x samples were each run in batch-triplicate
- the same batch SOP and dilution factor (250,000x) were used
- movie length = 2 seconds @ 11 movies/sample
- a different syringe was used for each of 10 samples
- the sample cell was rinsed between each sample injection

Total Time Elapsed to complete 10x triplicates = 47 minutes, 56 seconds = 2876 seconds for 10 samples **= 288 seconds/sample**

Table 2: Validation of Time-to-Result for Case 1 & 2 conditions

Case 2: ZetaView® Customer in Florida

Recently, a new customer of ours indicated that they ran 100 EV samples in a single day. While that isn't a typical workload, it does illustrate what is possible with our highly-efficient and automated NTA system.

The first question arises: Is this claim exaggerated? Secondly, is this possible if sample data is collected in triplicate? Table 2 shows the actual amount of time required to run 10x samples in triplicate with the ZetaView© system (288 sec/sample). In alignment with this directly measured sample rate, Table 3 shows our calculations for the total estimated time to run 100 samples in triplicate, in a single day, using a batch mode SOP with the ZetaView software. This task, while strenuous and probably requiring a few breaks, is clearly possible within less than 10 hours.

Case 2: Estimated time/sample to complete 100 samples in triplicate

2 sec per NTA movie x 11 movies = 22 seconds of data acquisition
+ additional time for instrument settling time between movies (18 sec)
+ time spent for tracking particles from NTA movies (30 sec)
= 70 seconds per single analysis

Triplicate Analysis:
+ pauses of batch processing after each of 3 analyses (10 sec)
+ time between each of 3 analyses to advance the sample (15 sec)
+ handling time & settling time for next sample injection (15 sec)
= ((70 + 10) x 3) + (15 x 2) + 15 = 3 analyses + 2 advances + 3x batch time
Estimated Total Time per Triplicate Sample = 285 seconds

Other Factors:
- use batch analysis with pumps (30 µ advance) or manual advance
- samples are pre-diluted, and a single SOP is used
- previous sample is flushed out NTA movie data is being tracked
- if we are realistic, there should be a 15 minute break between every 25 samples, in order to perform a sample cell cleaning

Analysis Time = 285 sec/sample x 100 = 7.9 hours
+ 45 minutes cleaning ***= 8 hours, 40 mins to complete 100 samples***

Table 3: ZetaView® Throughput: Case 2, Run 100 samples in 1 day

MRPS: Calculations via Vendor Recommendation

At a recent MRPS vendor webinar, it was suggested by the presenter that all three cartridge size ranges be used to characterize an EV sample. Run in triplicate, that is 9 cartridges per sample. Users of MRPS confirm that it takes at least 10 minutes per run for each cartridge, usually closer to 15. Based on these simple metrics, Table 4 shows our calculations of the cost and time spent to run 32 & 100 samples, which is 48 hours and 6 ½ days, respectively.

MRPS Calculations

Time to run 32 samples:
@ 10 minutes/cartridge and 3 cartridges/sample
= 10 x 3 x 3 = 90 minutes (run in triplicate)
for 32 samples = 2880 minutes **= 48 hours!**

Cost to run 32 samples:
$8/cartridge and 9 cartridges/sample
= $8 x 9 = $72/sample

32 samples = $72 x 32 = $2,304 (in 2 days)
running 100 samples = $7,200 (in 6½ days)

Table 4: Calculations for cost of MRPS usage for EV analysis

Both our time and cost estimates are likely very conservative, as we didn't factor in the handling time for changing cartridges, nor the time & cost for either clogged or failed cartridges (which require a do-over; customer report that both instances occur regularly), and we used a run-time estimate of only 10 mins rather than 15 minutes/cartridge.

Given the high cost to run samples in triplicate ($72), and the amount of time to do so (90 mins), it should be clear that the money saved by spending less on the MRPS system, relative to a ZetaView unit, is quickly spent within the first year of ownership, especially for high-rate users.

SP-IRIS: Time & Cost to Run 32 samples

In addition to the significant cost of buying an instrument in order to measure EVs with SP-IRIS, there is a substantial cost for the sample chips, as well as a greater amount of time needed to run samples, relative to using the ZetaView® system.

The steps required for data collection & analysis are described in detail in publications using SP-IRIS. Sample incubations are usually done overnight, with a standard time of 16 hours. Our cost estimates are based on the list price ($50) of a human tetraspanin chip (note: flexible chips for custom capture are priced >2x more), sold in kits of 16 chips. The reader system scans the chips @ Qty = 16/tray (older systems scan only 9/tray). Table 5 provides an estimation of cost & time for running 32 & 96 samples. While not as expensive to operate as MRPS units, this method is still costly relative to using the ZetaView system, given the initial cost of a reader system.

SP-IRIS Calculations

Time to run 32 samples:
16 hour (overnight) incubations of 16 chips/tray
1 hour dilution prep and deposit on-chip the day before
4 hours prep time & data collection the next day
Minimum 90 minutes of data analysis for final report
that's 6 ½ hours per tray x 2 **= 13 hours**

Cost to run 32 samples:
Chips come in kits of 16, so 32 samples is 2 full kits
That's 2 chip kits run in 2 days @ $800/kit (per website)
(minimum cost is $50/chip for human tetraspanin analysis)
(assumes known dilution factor, takes 3-4 chips to determine)
(minimum shipping charge = $80 per 2 kits)
(2 x $800) + $80 for 32 samples **= $1,680 (2 days)**
(6 x $800) + $240 for 96 samples **= $5,040 (1 week)**

Table 5: Calculations for cost of chip usage for SP-IRIS analysis

Summary

We've detailed how it is possible to run many EV samples within a short period of time, using a ZetaView© system. Further, our NTA systems use only low-cost consumables: standard oral syringes.

In the case of MRPS, while many consider the cost of the instrument inexpensive, relative to NTA, the cost of ownership soon becomes prohibitive for a lab that needs to run a significant number of EV samples.

For labs using SP-IRIS, the cost of operation would break the bank if many routine samples need to be run (@ 100/week). Realistically, most labs that use the SP-IRIS method also have an NTA system, as it is necessary to obtain both total particle counts along with more robust measurements of EV concentration in addition to studying captured populations of EVs.

Update

Recent EV analyses completed with the newest ZetaView® software (now more optimized for data collection efficiency) indicate that sample throughput is now > 25% faster than the results reported in this note.

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