The Cost of Liquid Handler QC – A Case Study

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Abstract

As with most laboratory instrumentation, automated liquid handlers should be assessed, verified and/or calibrated on a periodic basis. When considering an appropriate quality control (QC) method for the liquid handling instrumentation, one of the first questions asked is "How much does it cost to QC each system?" Oftentimes, the only aspect considered when contemplating total cost is the price of raw materials and necessary consumables. Very important, expensive components to obtaining complete quality control, such as time/labor, are often overlooked. As a result, incomplete, insufficient QC methods or processes are regularly created. This presentation attempts to summarize all consumable and labor costs associated with the current liquid handler QC method at an anonymous large pharmaceutical company. It then compares costs associated with other common liquid handler QC methods in an attempt to highlight the often misunderstood total cost of implementing and practicing a proper quality control process for liquid handling instrumentation.

Introduction

For the purpose of this presentation, the circumstances at one laboratory within a major (anonymous) pharmaceutical company are assessed. This group currently uses a combination of in-house developed fluorescence and single-dye absorbance methods for liquid handler QC to obtain tip-by-tip accuracy and precision performance data (**Table 1**). At each calibration event, five 96-channel and five 384-channel liquid handlers are evaluated using three replicate dispenses for three different target volumes per tip configuration. The per cycle cost for liquid handler QC is estimated using their current QC method. The information presented herein is based on documented sources compiled into a cost estimator model using a fully-loaded labor rate (labor + overhead) of $150/hour. Although $150/hour is used for this study, fully-loaded labor rates may range from $100/hour to greater than $200/hour. This poster also compares the costs for three common alternative QC methods (**Table 2**).

Additionally, this poster compares tangible and intangible aspects of each of the four dye-based QC methods (**Table 3**), which are often difficult to assign dollar values to and are not necessarily included in the total cost estimator model. For instance, operators and their pipetting technique are the largest sources of error when using hand held pipettes (refs 1 and 2) and some of the QC methods included herein require the preparation of a standard curve using a hand-held pipette. The cost of error and resulting economical impact due to error are not included in this study.

Table 1. Important Parameters of this Case Study

- Current QC method is combination of single-dye absorbance & fluorescence
- Imperative to have tip-by-tip accuracy and precision data
- Fully-loaded labor rate of $150/hour (labor + overhead)
- QC every liquid handler at each calibration cycle
- 10 automated liquid handlers
  - 5 x 96-tip
    - 3 target volumes-of-interest
    - 15 uL, 50 uL, 100 uL
  - 5 x 384-tip
    - 3 target volumes-of-interest
    - 2 uL, 5 uL, 20 uL
- Laboratory: what are the total costs per cycle for my QC method?
- Laboratory: do my costs compare to alternative QC methods?

Liquid Handler QC Methods: Overview of Four Methods

Laboratory's current in-house method
Combination of single-dye absorbance and single-dye fluorescence. The absorbance portion is used to determine precision and the fluorescence portion is employed to determine accuracy. The in-house developed, hybrid QC method consists of a preparing a standard curve for accuracy measurements. Refs 5 – 9.

Alternative method 1: single-dye absorbance
The single-dye absorbance method employs tartrazine dye at five different concentrations to create the standard curve with a handheld pipette. The same solutions are then used to test the validity of the curve. The solutions and standard curve are prepared each time the QC testing is performed. Standard, off-the-shelf microplates are used for these steps. Refs 5 – 9.

Alternative method 2: single-dye fluorescence
The fluorescence method uses eosin dye at one concentration. Three volumes are manually dispensed into a microplate to generate the standard curve. The same dye solution is then used to validate the curve. Standard microplates are used. Due to the low number of data points on the curve, pipetting variability must be minimized in order to ensure acceptable performance from one calibration event to the next. Refs 5 – 9.

Alternative method 3: Artel MVS® Multichannel Verification System
The Artel MVS is a dual-dye, dual-wavelength volume measurement system based on ratiometric photometry. The absorbance-based method employs factory manufactured dye solutions. The basis of the dual-dye method resides in using two different types of solution: (a) sample solution, which contains known concentrations of two dyes (dye 1 and dye 2); and (b) diluent solution which contains a known concentration of dye 2, but at the same concentration as in the sample solution. Because the diluent contains the same known concentration of dye 2 as does the sample solution, no dilution of this dye occurs when diluent is added to sample. The system simultaneously measures accuracy and precision on a tip-by-tip basis with no need for preparing standard curves or solutions. Measurement results are traceable to international standards and operator pipetting skill is not required. Refs 10 – 11.

Liquid Handler QC Methods: Importance

Regardless of the type of volume verification method employed to evaluate liquid handler performance, it is of the most importance that the methodology is scientifically-based, analytically-implemented and executed properly. If the volume verification method is not well thought out or properly implemented, a false sense of liquid handler performance may result. Volume verification methods used for instrument qualification should also be performed in the same environment (e.g., temperature, pressure, humidity) that the liquid handler operates in (refs 3, 4). Transport of components and materials/parts handling may introduce more error in the instrument qualification process. When handling microtiter plates with reagents, there is a possibility of evaporation and loss of reagent, which may also introduce error into the measurement that should not be attributed to the performance of the liquid handler.

Liquid Handler QC Methods: Considerations

There are pros and cons associated with each independent QC method. The goal of this presentation is not to describe each method in detail, but rather give an overview of the methods, associated estimated totals costs, and also discuss some of the intangible topics that do not get rolled into the cost of QC. Due to this pharmaceutical laboratory's need for obtaining both accuracy and precision information on a tip-by-tip basis for their 96- and 384-tip liquid handlers, some QC methods, such as gravimetry, were not included in this analysis. For tip-by-tip volume transfer statistics for these specific 10 liquid handlers, it would be cost prohibitive to employ gravimetry as the QC method due to the amount of time required by the technician and the amount of time each liquid handler would be off-line. For instance, the 96-tips can dispense liquid into 96 individual containers such as test tubes and the tubes are individually weighed on the balance. This process can take hours to days, especially if at least three replicates are used for statistically determining performance values per tip.

The pharmaceutical laboratory's current QC method, as well as the three different alternative liquid handler QC methods, are compared. All four QC methods are spectroscopic approaches where either absorbance or fluorescent dye-based test solutions are used to asses liquid handler performance. In all cases, a dye solution is used to generate a measurable light signal that can be used in some fashion to determine the precision and/or accuracy of a volume transfer within a microtiter plate. In many respects, the single-dye absorbance and fluorescence methods are carried out in a similar fashion. They both require a plate reader, one or more solutions are prepared (with and without dye), and in most cases a buffer solution is added before or after the target volume in the microtiter plate to provide a total working volume in each well. There must be skill and patience in the weighing of dye powder, filtering insoluble components from the solutions and each method should employ necessary controls, such as using an appropriate baseline subtraction (e.g., zero measurement) and accounting for plate reader drift. When using a single-dye fluorescence or absorbance method, the volume of buffer used to fill the well to a total working volume needs to be accurate (i.e., known), otherwise the uncertainty in the buffer volume cannot be separated from the uncertainty in the target volume. The types and quality of the plates can also have a large effect on the liquid handler assessment, especially if plates and/or lots of plates are interchanged during the evaluation process. Additionally, it is very important that dye solutions in the wells are efficiently mixed, otherwise the QC method may give a false-sense of liquid handler performance (good or bad).

For qualifying liquid handlers and/or assessing their performance, it is critical to measure both the accuracy *and* precision of each target volume transfer. The precision of any tip of a liquid handler can be easily determined by measuring the standard deviation of the light signal measured across multiple wells of a plate of solution dispensed by each tip. For determining accuracy, a standard curve can be generated and used to measure accuracy of a liquid handler on a tip-by-tip basis. To prepare a proper standard curve, a calibrated pipette should be used by a skilled operator to dispense the desired target volumes of dye solution into a microplate. The buffer, or diluent, also must be accurately added to the target volume. If the buffer is not accurately and reproducibly dispensed, the calibration curve can be seriously flawed because the dye could be diluted differently in different wells. A dual-dye approach is protected against the quantitative addition of buffer to the target volume (see below for alternative QC method 3 – MVS). With the standard curve, the measured optical signal is plotted versus the calibrated volume of the pipette or syringe and from this data, a "best fit" equation is generated over the volume range of interest and is subsequently used in determining the accuracy of liquid handler for a specific target volume. Care and skill must be practiced in the generation of the standard curve, e.g., in the preparation of the dye solution (weighing dye powder, filtering, protecting from light, labeling) as well as in the use of the calibrated pipettes. Because there are substantial differences from operator-to-operator when using calibrated pipettes, standard curves prepared by different technicians could differ significantly. The same might also hold true for different dye solutions prepared by different technicians where the solutions and/or quality of the solutions, and the associated documentation (e.g., SOPs or lab notebooks) could be different from person-to-person or lab-to-lab.

Table 2. Total Costs Per Calibration Cycle for Four Liquid Handler QC Methods*

	Current QC Method: Combination of Single-dye Absorbance & Fluorescence	Alternative QC Method 1: Single-dye Absorbance	Alternative QC Method 2: Single-dye Fluorescence	Alternative QC Method 3: Artel MVS®
Consumables (chemicals/dyes/plates)	$920	$665	$535	$1,830
Hardware Setup	$25	$13	$13	$18
Software Setup	$0	$0	$0	$13
Solution Prep	$350	$190	$165	$0
Standard Curve Prep	$100	$100	$100	$0
Solution Dispensing	$1,145	$850	$850	$850
Plate Prep (mixing/centrifuge)	$1,125	$375	$750	$525
Plate Measurement	$750	$375	$375	$690
Data Calculation	$1,125	$750	$750	$0
Report Prep	$1,125	$750	$750	$0
Sum	**$6,665**	**$4,068**	**$4,288**	**$3,926**

* refer to **Table 1** for calibration cycle parameters

Table 3. Tangible and Intangible Aspects of the QC Methods

	Current QC Method: Combination of Single-dye Absorbance & Fluorescence	Alternative QC Method 1: Single-dye Absorbance	Alternative QC Method 2: Single-dye Fluorescence	Alternative QC Method 3: Artel MVS®
Multiple dye solutions need to be *prepared* to cover entire volume range	√	√		
Dyes used for testing are available from multiple vendors	√	√	√	
A standard curve must be prepared	√	√	√	
Manual pipetting skill is required	√	√	√	
Standard curve might be different if prepared by different operators or on a different day relative to QC testing	√	√	√	
If tips on manual pipette (for standard curve prep) are of poor quality or of a markedly different type than employed on liquid handler under test (i.e., sterile vs. non-, filter-based vs. non-, low-retention vs. non-, etc.) results could be affected	√	√	√	
The addition of buffer is critical (quantitative buffer addition required)	√	√	√	
Plate reader fluctuations/noise/drift may affect measurement results	√	√	√	
Variations in plate dimensions (well-to-well) vary from one lot to the next and may impact results	√	√		
Inefficient dye mixing in microplate may cause false-sense of liquid handler performance	√	√	√	√
Output report is manually generated	√	√	√	
Output report and data are automatically archived				√
Measurement results are traceable to international standards				√
The report-generating software conforms to 21CFR Part 11 with audit trail, e-signatures, protected databases				√
Accuracy and precision measured on tip-by-tip basis	√	√	√	√
QC method *is transferrable* between operators/labs (i.e. method is independent of operator or location of testing event)				√
Dye solutions have known stability and known shelf-life				√

Discussion & Conclusions

In order to make an accurate comparison, all costs are estimated based on the requirement for **tip-by-tip accuracy and precision data** and other parameters summarized in **Table 1**. It is understood that the parameters of the cited referenced methods may not be exact replications, but rather representations, of those used for liquid handler QC. From the cost estimator models and based on the fully-loaded labor rate of $150/hr, the current QC method, which is a combination of both absorbance and fluorescence steps for the ten multichannel liquid handlers in this laboratory, is the most expensive when all costs are considered. The three alternative QC methods are all within $400 of one another with most of cost for the two single-dye approaches coming from labor and the most of the cost for the dual-dye MVS coming from consumables. Note, as the fully-loaded labor rate increases, the total cost of each method changes. Those methods that are more labor-intensive, such as the single-dye methods, will be highly effected, while methods that are less time consuming will experience very little effect. Technician pipetting skill is a must for all methods except the MVS approach, which is also the only method that is easily transferrable between labs, groups, or location due to it being a standardized measurement system. QC requirements can be unique for every liquid handler application making it necessary for every laboratory to consider many parameters when selecting the QC method that best meets their needs. As budgets are more and more constrained, understanding the true cost of the selected QC method is paramount. In order to make a complete assessment of those costs, a detailed analysis including costs of materials *as well as* labor and overhead must be performed and the value of equipment down-time must be considered. Not included in this presentation is the cost of equipment (pipettes, plate readers, etc.) or the economic impact associated with equipment malfunction, downtime and delays, which may be the largest "hidden" cost of all.

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