A112 Is Your Automated Liquid Handler Working for Your Assays? – Uncovering Errors, Understanding Device Behavior, and Optimizing Methods

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After attending this presentation, the attendees will appreciate the need to check, calibrate, and/or verify the volume transfer performance ("device behavior") of automated liquid handlers employed for important laboratory tasks.

This presentation will impact the forensic community by focusing attention on assays that are performed with automated liqid handlers. Without checking these systems for performance metrics, the assay results could, in some cases, be unknowingly flawed where the results cannot be trusted - or hold up in court.

The focus of this presentation is to highlight the need of ensuring quality in important assays performed with automated liquid handlers. Nearly all assays performed within a laboratory are volume-dependent. In turn, all concentrations of biological and chemical components in these assays, as well as the associated dilution protocols, are volume- dependent. Because analyte concentration is volume-dependent, an assay's results might be falsely interpreted *if* liquid handler variability and inaccuracies are unknown *or* if the system(s) go unchecked for a long period. No one wants to perform assays that could essentially produce meaningless results. If liquid handlers are properly employed (with the right methods/materials for the specific assay) *and* they are regularly assessed for performance, they can be powerful systems for lowering costs, increasing throughput, and avoiding errors associated with manually-pipetted methods. It is imperative, therefore, to quantify the volumes transferred with an automated liquid handler, especially for the specific automated methods that are used to perform the assays. Measuring and knowing the exact volumes transferred, for specific and/or routine methods, will inherently lead to confidence in the experiment, *i.e*., the results can be trusted.

As presented herein, a case-study is shared where a real time polymerase chain reaction (RT-PCR) assay was being transferred from the bench (using a manually pipetted method) to an automated liquid handler. Many assays, such as RT-PCR assays, depend on accurate volume delivery. The technician was observing acceptable results with the manual assay, but as the assay was transferred to the automated pipettor, the results could not be repeated and were deemed unacceptable, *i.e*., the liquid handler was producing errant results for the same assay that worked with a handheld pipette. In brief, the automated liquid handler was delivering a different volume compared to the manual method and ultimately, it was blamed for performing poorly. It is often the case that assays are initially performed on the benchtop using handheld pipettes before they graduate, or transfer, to an automated liquid handler. During the transfer process; however, the manual assay should be directly compared to the automated assay for consistencies in pipetting performance. As discussed, this RT-PCR assay transfer was finally successful after simply comparing the volume transfer performance of the handheld pipette vs. the automated liquid handler.

This presentation focuses on the importance of understanding liquid handler behavior for forensic assays. To understand and assess the liquid handler performance, the MVS® Multichannel Verification System was employed. The MVS measurement results are traceable to NIST and the measurement methodology follows international guidelines (ISO 8655, Part 7). By using this system, the RT-PCR assay method was successfully transferred from the bench to the automation. Additionally, the measurement system was used in numerous forensics laboratories to assess liquid handling behavior when running specific methods. For instance, it will be presented how the liquid handling steps were measured, understood, and/or optimized for numerous assay experiments, including: (1) on-board mixing efficiency; (2) tip-to-tip reproducibility; (1) finding a bad "tip-in-the-box" in a newly opened tip box; (4) highlighting the differences between accuracy and precision; (5) comparing individual volume transfers over multi-sequential dispenses; (6) optimizing an automated method for a specific target volume; and, (7) directly comparing performance between liquid handlers from multiple locations.

Liquid Handling Error, RT-PCR Assay Transfer, Liquid Handler Behavior

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