A simple method for validation and verification of pipettes mounted on automated liquid handlers

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A simple method for validation and verification of pipettes mounted on automated liquid handlers

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INTRODUCTION

Automated liquid handlers (ALHs) are increasingly used to improve throughput, pipetting accuracy and prevent occupational injuries to the technical staff due to intensive manual pipetting[1]. Standardized procedures for calibration of standard piston operated pipettes exist and many laboratories calibrate their pipettes with defined intervals[2]. However, less standardized is the routine validation and verification of ALHs, though suggestions to how it should be performed exist[3]. Commercial solutions have also been introduced[4].

MATERIAL AND METHODS

A 7-step serial dilution of Orange G was prepared manually in quadruplicates in a flat bottom 96-well microtiter plate (BD Falcon) by means of calibrated pipettes (column 9-12). This was used as a standard row. Each pipette of the liquid handler (1 up to 8) dispensed a selected volume (1 to 200µL) of Orange G eight or more times into the wells of the microtiter plate. All wells contained a total of 200µL liquid. The optical density (OD) was read at 490 nm, and the dispensed volume of each pipette was calculated based on a plot of volume and OD of a known set of Orange G dilutions. Finally, the percent inaccuracy (%d) and the imprecision (%CV) of each pipette was calculated.

Figure 1. Plate layout. A standard row is included on each plate.

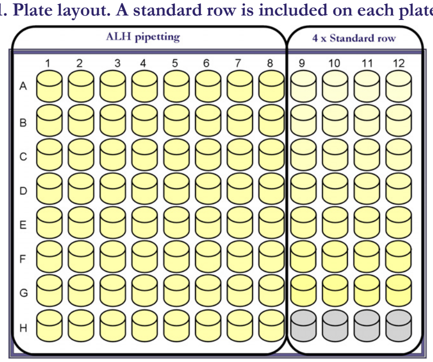
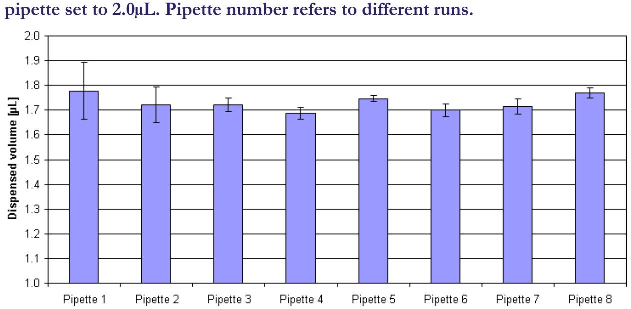


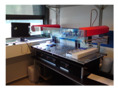
Figure 2. Volume verification using manual pipetting and a conventional pipette set to 2.0µL. Pipette number refers to different runs.

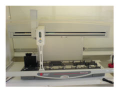


PROCESS FLOW

The process is composed of few and simple steps. The same stock solution may be used for verification of multiple ALHs. A calibrated ALH may be used to prepare the standard row. Initial calibration was performed with manually pipetted standard rows.

- Prepare OG stock slution
- Prepare standards for standard rows
- Prepare standard rows
- Distribute water to rows 1-8
- Transfer plate to ALH
- Distribute OG in rows 1-8
- Read absorbance and calculate distributed volumes





RESULTS

Using pre-defined acceptance criteria, each pipette on each ALH was then either approved or rejected. Rejected pipettes were either repaired or the volume deviation was compensated for by applying a calibration curve in the liquid handler software. We have implemented the method on a Sias Xantus, a MWGt TheONYX, four Tecan Freedom EVO 150, a Biomek NX Span-8 and four Biomek 3000 robots.

Figure 3. Two different volume verification runs both using 50µL as target volume on a 4 channel fixed tip MWG TheONYX robot.

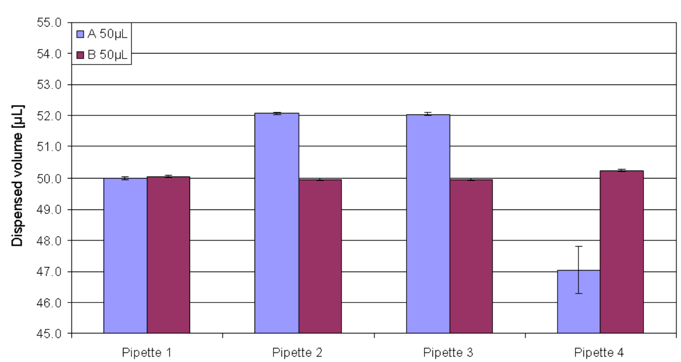


Figure 4: Two different volume verification runs both using 5.0µL as target volume on an eight channel fixed tip Sias Xantus robot. Run A showed unacceptable pipetting. Run B following calibration showed acceptable pipetting.

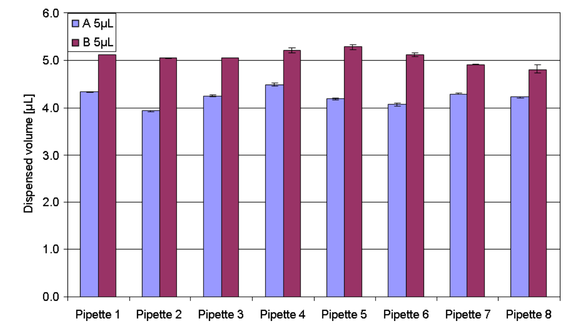
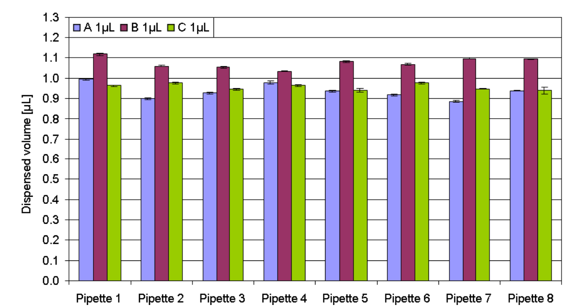


Figure 5. Three different volume verification runs all using 1.0µL as target volume on a Biomek NX span-8 using disposable tips. Run A and Run B show either too little or too large volume dispensed. Run C shows acceptable pipetting.



CONCLUSIONS

We have set up and implemented a simple solution for the continuous verification of pipettes mounted on automated liquid handlers as necessary for accredited work under the international laboratory standard ISO 17025. The method is cheap, simple and easy to use for aqueous solutions, but it requires a spectrophotometer that can read microtiter plates. The method can be used with both disposable tips, fixed tips as well as manual pipetting.

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