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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 (columns 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.

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.

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.

REFERENCES