

Validation of VERSA Mini PCR Setup Workstation for High Throughput PCR

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I. Abstract

As the majority of laboratory research processes are liquid based, automated liquid handling systems play a significant role in genomic studies. The primary applications in these fields include isolation, normalization, amplification, purification, cloning, and analysis of DNA and RNA. Repetitiveness of tasks and sample numbers above a certain limit make manual performance difficult and time-consuming. As a solution to this problem, efficient liquid handling workstations for many of these applications are now available.

The high throughput features of these systems, which reduce cost while improving speed and quality, are critical concerns. Moreover, specific applications need specific features from a liquid handler. The throughput, miniaturization of liquid volumes, precision and accuracy, sterility, prevention of cross contamination, and footprint size are important factors for decision-makers. In addition, hardware-software compatibility is important to allow the researcher flexibility to customize the protocol for better control and performance. Choosing an appropriate system for the application will enable the researcher to analyze in parallel large numbers of samples with precision and accuracy.

Validation of Aurora Biomed's VERSA Mini PCR Setup Workstation for genomic applications will be presented.

II. Introduction

Ever since polymerase chain reaction (PCR) was first described in 1985, the technique and its applications have gone through many rounds of their own amplification. PCR is now a valuable tool in academic, diagnostic, medicolegal and various other laboratories where it is used routinely for a variety of tasks. Although this technique is simple, it requires accuracy in aspirations and delivery of precious reagents and volumes.

The modern genomics labs are looking for a high-throughput, quality-data workstations to perform PCR reactions at a reduced cost. PCR reaction setup is a tedious and time consuming process as it requires the mixture of multiple reagents in specific ratios to achieve the proper master mix for amplification. Moreover, the exact volumes of the reagents and buffers depend upon the total number of samples to be amplified and the desired final concentration of sample DNA. Automation of such procedures eliminates both human error and contamination problems generally associated with manual process. To provide an automated solution to such a cumbersome protocol, Aurora Biomed's VERSA Mini PCR Setup Workstation was developed for the preparation of reagents and samples used in an amplification protocol in 96-well format.

III. Objectives

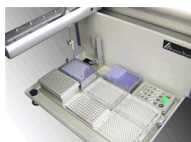
As modern genomics labs are always looking for high-throughput quality data, this validation was carried out with the following objectives:

- Minimum volume aspiration and delivery
- Minimum volume of master mix (MM) preparation
- Minimum volume of DNA sample
- Ratio of different reagents in the preparation of MM
- Ratio of DNA / MM
- Cross contamination-free reagent delivery
- Stability of MM for entire duration of 96-well plate

IV. Materials and Methods

1. VERSA Mini PCR set-up Workstation was used in the validation studies.

VERSA Mini PCR Set-up Workstation



2. To determine accuracy of aspiration and delivery, 1 μ L of a concentrated blue liquid was dispensed into 1 ml of deionized water. Absorption of the dye mix was determined by spectrophotometry at 632nm. This procedure was repeated for ten replicates using both VERSA and manual pipettors.

3. Accuracy in content/volume delivery for VERSA was carried using atomic absorption spectroscopy (AAS) to measure the concentration of two analytes, Rubidium (Rb^+) and Lithium (Li^+). Rb^+ and Li^+ were added to Taq polymerase reagent, and to the PCR buffer, respectively. For 50 μ L reaction volume, mock Taq polymerase (0.5 μ L), PCR buffer (15 μ L), and water (32.5 μ L) were aspirated from respective vials on the cooling block at 4°C and dispensed into MM vial. The MM was prepared for 8, 16, 24, 32, 40, 48, 56, 64, 72, 80, 88, and 96 samples. The amount of Rb^+ and Li^+ in the MM was determined by AAS in each of these samples.

Similarly, in separate experiments, Rb^+ and Li^+ were added to DNA samples and MM respectively, to check the amount and ratio of these two analytes in the reaction wells.

4. To check for any cross contamination of DNA samples, a test was designed to deliver 10 μ L of DNA samples containing 5.4 mM of Rb^+ alternating with samples having no Rb^+ . Upon distribution of these samples to the target well dispensed with 40 μ L of MM containing Li^+ , the wells were analyzed for Rb^+ and Li^+ content. Since 5.4mM Rb^+ solution was 23000X greater than the detection limit of AAS, cross contamination of the samples could be detected in the well surrounded by Rb^+ containing wells.
5. PCR setup was carried following volume delivery as shown in Table 1. The 96-well plates were run in MyCycler (BioRad Labs) under conditions given in Table 2.

Table 1: Reagent volumes for the preparation of MM

| No | Reagent | Vol (μ L) |
|----|---------------------|----------------|
| 1 | Nuclease free water | 6 |
| 2 | Forward primer | 1 |
| 3 | Reverse Primer | 1 |
| 4 | ReadyMix | 10 |
| 5 | DNA template | 2 |
| | Total volume | 20 |

Table 2: Thermocycler conditions

| Step | Temp ($^{\circ}$ C) | Time (min) | Cycles |
|--------------------------|----------------------|------------|--------|
| Initial denaturation | 95 | 2 | 1 |
| Denaturation | 95 | 1 | 40 |
| Annealing | 55 | 1 | |
| Extension | 72 | 1 | |
| Final Extension | 72 | 5 | 1 |
| Storage (short duration) | 4 | Hold | |

V. Results

A. What is the accuracy of the aspirating and dispensing capabilities of small reagent volumes?

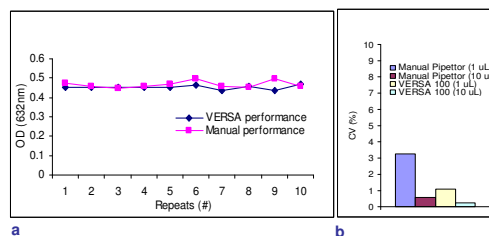


Figure 1. Since precision and CV% values are dependant upon the technique used for validation², VERSA performance was compared with manual performance. In this case, the concentration of samples was determined by spectrophotometry. (a) OD values were calculated for ten replicates using VERSA, and a manual pipettor. Comparative profile of VERSA and manual pipettor performance for 1 μ L delivery are shown. (b) Comparative CV% for 2, and 10 μ L delivery was lower for VERSA than manual pipettor (1 μ L=1.07% vs. 3.2%, 10 μ L 0.23%, and 0.58%, respectively).

B. Was the ratio of Taq polymerase to primers & dNTPs in the MM, OR the ratio of DNA sample to the MM comparable between VERSA performance and manual performance?

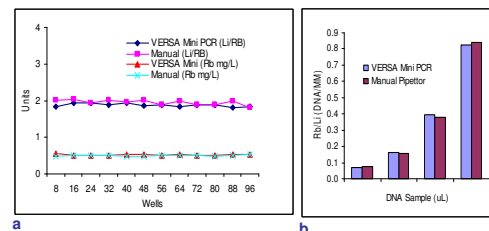


Figure 2: The results indicated that delivery of reagents is comparable between VERSA and manual pipetting. (a) Reagent delivery with VERSA and manual pipettor for 8 to 96 wells. The reagent delivery and the ratio of reagents like Taq polymerase to PCR buffer and water indicated by Li^+/Rb^+ was quite uniform in both the automated and manual performance. Automated performance appeared better than manual beyond 24 well volumes. This also shows that small volume of MM (for example 8-reactions) can be accurately prepared. (b) The comparison of automated delivery of DNA samples and its ratio with MM containing Li^+ in the reaction wells was comparable with the manual pipettor for 1, 2, 5 and 10 μ L of the DNA samples containing Rb^+ .

C. Does VERSA automation lead to any cross contamination of DNA samples?

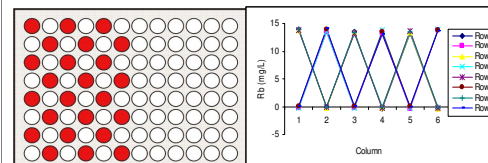


Figure 3. (a) The design of DNA samples in the plate where DNA sample contained either 5.4 mM of Rb^+ (red wells) or no Rb^+ (white wells) and 10 μ L of the sample was aspirated with VERSA and delivered to the reaction plate in the same order. (b) The data presented indicated no cross contamination among the samples.

D. How stable was the master mix if distributed to entire plate prior to the addition of DNA samples?

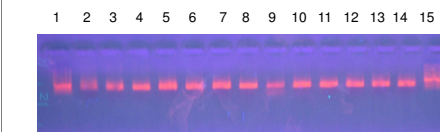


Figure 4: Amplification of 20 μ L DNA carried with PCR Core System-II kit using BioRad MyCycler (96 well). Lane 1 & 15: DNA Ladder, lane 2-13: automated reaction set up (from different positions in the 96-well plate). Lane14: manual reaction set up.

VI. Conclusion

1. The results were consistent and clean, indicating accurate and precise volume delivery of samples (as low as 1 μ L).
2. The ratio of Taq polymerase to other components in the master mix was quite uniform from 96 to 8-reaction wells.
3. The performance of VERSA indicated by ratio of DNA sample to the master mix in the reaction well was comparable with manual.
4. The delivery of the samples utilizing the automatic tip changer generated no cross contamination among samples.
5. The master mix is stable even if distributed to the whole 96-well plate prior to the addition of the DNA sample.
6. The VERSA Mini PCR Setup Workstation is suitable and appropriate to automate PCR Setup experiments.

VII. Acknowledgements

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VIII. Reference

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2. Rhode et al.: An Improved Method for Checking HTS/uHTS Liquid-Handling Systems. Journal of Biomolecular Screening, 2004; 9(8):726-733.