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Automation of a One-Step, Real-Time PCR-Based DNA Quantitative Kit for the Diagnosis of HBV

Automation of one-tube or one-step real-time PCR-based molecular diagnostics has distinct advantages for sample processing in preparative work. It saves time and effort, improves processing capacity, eliminates human error and ensures batch consistency of reagents and samples in the detection and diagnostic process of clinical samples. The VERSA 10 workstation (Aurora Biomed, Inc., Vancouver, B.C.) was validated for automated use for a Hepatitis B viral (HBV) DNA quantitative fluorescent diagnostic kit (Sansure Biotech, Changsha, Hunan, China).

Results show that the automated operation was performed with consistency, appreciable throughput and accuracy with no detectable cross-contamination. Ninety-six samples can be processed within 25 minutes using a total of 108 50- μ L tips with one click on the software interface. The system includes a high-efficiency air filter hood, an automated shaker and liquid handling.

Introduction

Hepatitis B is a significant global health problem: more than 780,000 people die each year due to complications associated with the virus. Its prognosis and diagnosis have been primarily based on the immunodetection of HBV surface antigen. The lack of detectable antibodies in human patients has necessitated the use of nucleic acid amplification-based assays for their sensitivity, specificity and tolerance of sequence variation.^{1,2} In the last few years, the field of HBV molecular diagnostics and prognosis has included such tools as real-time PCR.³ A one-tube or one-step real-time detection and quantification method for HBV DNA using nucleic acid sequence-based amplification further advances the benefits of these methods.^{4,5} Sample processing and amplification are performed in one tube and the need to heat or centrifuge samples is eliminated.



Figure 1 – VERSA 10 workstation with HEPA/UV enclosure.



Figure 2 – Hepatitis B viral DNA quantitative fluorescence diagnostic kit.

Materials and methods

The validation of the kit was conducted on the VERSA 10 workstation (Figure 1) with four-channel liquid handling configuration as follows:

1. 48 samples including 42 different concentration HBV serum samples, four standards, one negative and one positive control.
2. Hepatitis B viral DNA quantitative fluorescence diagnostic kit, 48 tests (Figure 2).
3. Software.
4. The following steps were automated in a PCR plate:
 - a. Addition of samples and reagents
 - b. Addition of 5 μ L of lysis buffer: addition of 5 μ L of sample and 40 μ L of master mix and primer sets
 - c. Sensitivity test: 1.96 e+05 IU/mL of hepatitis sample, of high concentration, was diluted to 1.96 e+02 IU/mL low concentration to determine whether automation could meet kit performance requirements while dealing with low-concentration samples

- d. A cross-contamination test was designed as shown in the plate map (Figure 3). Mixing of the contents in the plate was done at 600 rpm for 10 minutes on the plate shaker housed on the deck
5. The manual process was carried out in parallel to the automation for comparative evaluation as per the manufacturer's directions.
6. The plate was manually transferred to the SLAN real-time PCR thermocycler for amplification (Hongshi, Shanghai, China).

Results

Sensitivity

The objective of this experiment was to examine whether the workstation can achieve kit performance requirements while dealing with low concentration samples. The amplification curves in Figure 4a and b indicate that the VERSA 10 procedure achieves better performance than the manual method when handling low concentration samples. As seen in Figure 4, automated sample preparation resulted in more consistent cycle threshold (ct) values and amplification curves, accounting for

Sample processing and amplification are performed in one tube and the need to heat or centrifuge samples is eliminated.

	1	2	3	4	5	6
A	H	NTC	H	NTC	H	NTC
B	NTC	H	NTC	H	NTC	H
C	H	NTC	H	NTC	H	NTC
D	NTC	H	NTC	H	NTC	H
E	H	NTC	H	NTC	H	NTC
F	NTC	H	NTC	H	NTC	H
G	H	NTC	H	NTC	H	NTC
H	NTC	H	NTC	H	NTC	H

Figure 3 – Plate map for testing cross-contamination high-concentration sample (H) and negative sample (NTC).

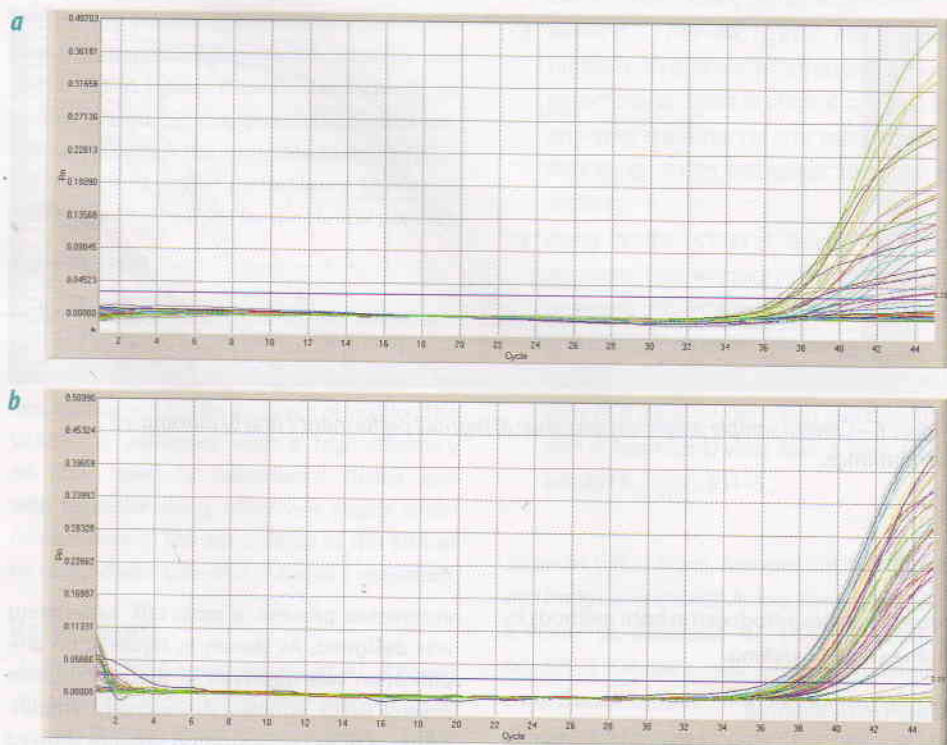


Figure 4 – Manual (a) and automated (b) comparison test for 48 samples.

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by Jeanelly Hunt, MS, MBA, Web Producer,
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PCR-BASED DNA QUANTITATIVE KIT *continued*

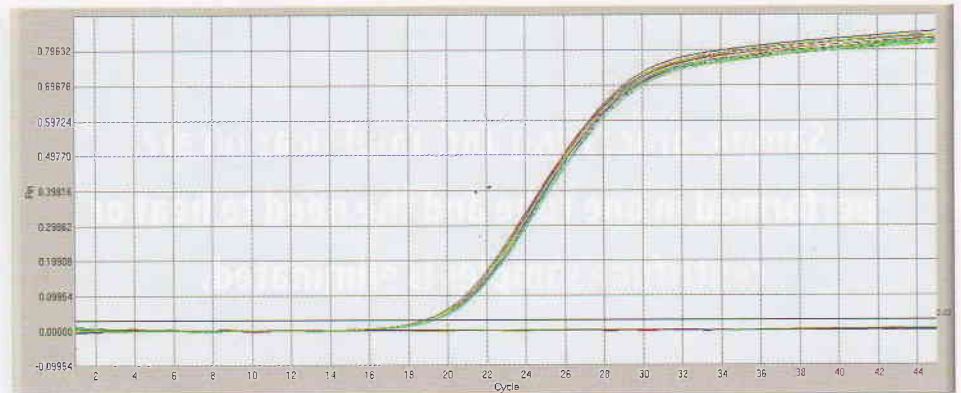


Figure 5 – Cross-contamination sample amplification curve.

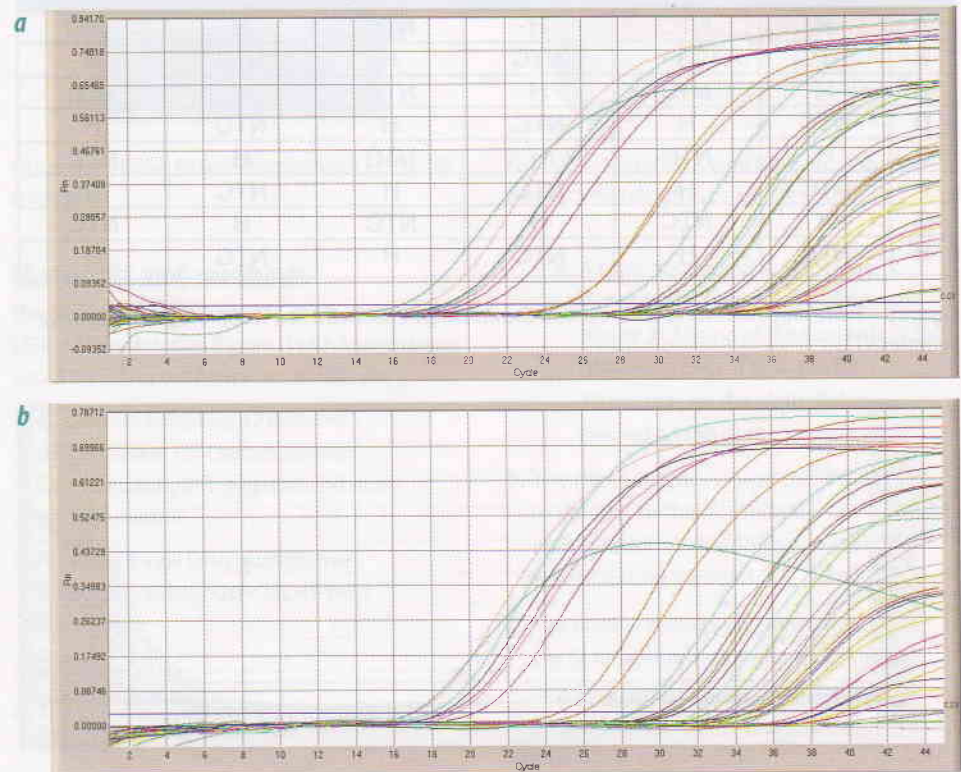


Figure 6 – Clinical sample amplification curve: a) manual performance and b) automated performance.

inconsistencies introduced in both methods by human liquid handling.

Cross-contamination

Since it was necessary to determine whether there is any cross-contamination during the

automated process, a cross-talk experiment was designed. As shown in Figure 5, no amplification was observed in the no template control (NTC) samples. Additionally, amplification of high-concentration samples showed very good repeatability.

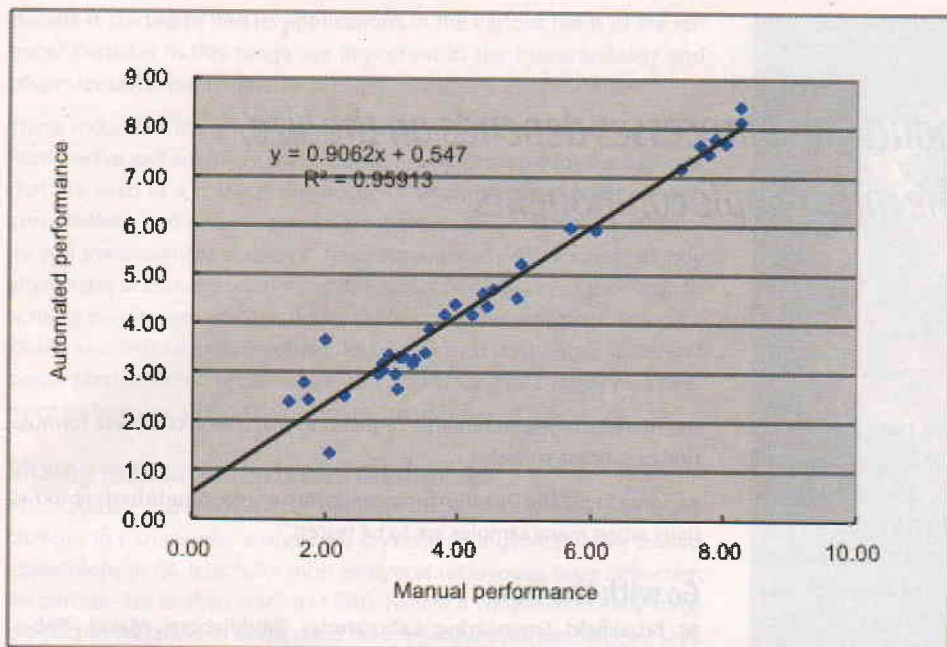


Figure 7 – Quantitative linear correlation curve of automated ($R^2 = 0.97$) and manual performance ($R^2 = 0.96$).

Clinical sample test results

The purpose of this experiment was to test the performance requirements of the kit on VERSA 10 for dealing with clinical samples. Forty-eight samples were run in both automated and manual methods. The amplification curves and comparative quantitative analysis are presented in Figures 6 and 7, respectively. As shown in Figure 7, the quantitative linear correlation curve for the automated samples ($R^2 = 0.97$) confirms similar performance to manual performance ($R^2 = 0.96$) for the clinical samples.

Conclusion

Automation offers higher consistency, throughput and accuracy than the manual process; can also provide time and energy savings; and offers confidence in the accuracy of results. The VERSA 10, equipped with a high-efficiency air filter hood, an automated shaker and with liquid handling, effectively avoids cross-contamination. The automation of this kit can be realized with one-click automatic operation.

References

1. Datta, S.; Chatterjee S. et al. Recent advances in molecular diagnostics of hepatitis B virus. *World J. Gastroenterol.* **2014**, 20(40), 14,615–25.
2. Yates, S.; Penning, M. et al. Quantitative detection of hepatitis B virus DNA by real-time nucleic acid sequence-based amplification with molecular beacon detection. *J. Clin. Microbiol.* **2001**, 39(10), 3656–65.
3. Bennett, S.; Harvala, H. et al. Rapid simultaneous detection of enterovirus and parechovirus RNAs in clinical samples by one-step real-time reverse transcription-PCR assay. *J. Clin. Microbiol.* **2011**, 49(7), 2620–4.
4. Jiang, W.; Yu, H.T. et al. Quantification of Hantaan virus with a SYBR green I-based one-step qRT-PCR assay. *PLoS One* **2013**, 8(11), 81,525–31.
5. Kodani, M.; Martin, A. et al. One-step real-time PCR assay for detection and quantitation of hepatitis D virus RNA. *J. Virol. Methods* **2013**, 193(2), 531–5.

Sikander Gill, Li Wenji, Rajwant Gill, Nick Bandy and Dong Liang are with Aurora Biomed Inc., 1001 E. Pender St., Vancouver, B.C. V6A 1W2, Canada; tel.: 604-215-8700; e-mail: info@aurorabiomed.com; www.aurorabiomed.com. Fan Xu and He Bin are with Sansure Biotech, Changsha, Hunan, China; www.sansure.com.cn

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