A RAPID MICRO POLYMERASE CHAIN REACTION SYSTEM (GenSpector® Micro PCR) FOR *HEPATITIS* B VIRUS DNA DETECTION

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Abstract

This paper presents a rapid micro PCR (polymerase chain reaction) system (GenSpector® Micro PCR) for the application of *Hepatitis* B virus (HBV) DNA detection. Silicon micromachining technology has been utilized to miniaturize the conventional PCR system. Each module in the system has been installed with an optic system that makes real time in-situ monitoring possible, as well as a built in computer system complete with user-friendly touch screen access. In addition, newly developed electronic temperature controls have cut down response time, making the system faster than ever before. Also, the system enables one or more operators to apply for qualitative or quantitative PCR. Thus, the GenSpector® Micro PCR system will have various applications in nucleic acid point-of-care-tests.

Keywords: PCR, Micro PCR, HBV DNA, Thermal Cycler, Clinical Diagnostics

1. Introduction

This paper presents a rapid micro PCR (polymerase chain reaction) system (GenSpector® Micro PCR) for the application of *Hepatitis* B virus (HBV) DNA detection [1]. Silicon micromachining technology has been utilized to miniaturize the conventional PCR system for the application of point-of-care-test (POCT). The system has shown excellent clinical diagnostic results for HBV DNA: 93.8% in sensitivity, 96.4% in specificity, 95.1% in accuracy, and 91.7% in reproducibility.

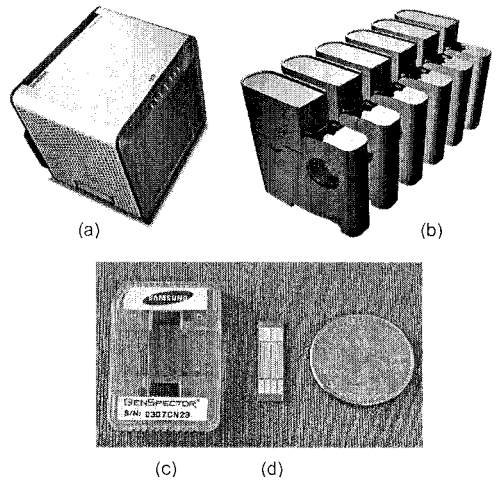

Figure 1. A GenSpector® Micro PCR system (a), 6 thermal cycling modules (b), a Micro PCR chip with plastic housing (c), and a silicon-based heater/sensor plate (d).

2. System Design

A micro PCR system, shown in Fig. 1, consists of six individual thermal cycling modules and a computing unit. A plastic packaged silicon micromachined PCR chip is inserted in each module. The PCR chip is comprised of a micro chamber on a silicon substrate side, and an optical window, an inlet and an outlet on a glass substrate side. The module has a silicon-based heater/sensor plate, a cooling fan, an optic unit for light excitations and emissions, an embedded microprocessor and a computing circuit board. Each module is capable of independent thermal

cycling and in-situ monitoring of the micro PCR chips, as shown in Fig. 1. Independent PID (proportional integral derivative) control by an embedded microprocessor in each module can give an excellent temperature accuracy of less than 0.5° for PCR.

The computing unit in the PCR system is basically a PC including a 15.1" touch screen TFT-LCD, a 2.5" 30GB hard disk drive, 4 USB drive ports, an internet accessible LAN, and a Windows 2000 operating system. When thermal cycling is not in progress the system can be used as a regular computer, complete with LAN connection and network sharing.

Fig. 2 shows a micro PCR software, which enables to define and simultaneously conduct separate PCR experiments, with a set of thermal cycling protocols. Additionally, thermal and optical data can be in-situ monitored, and graphs of temperature, amplification, and melt curves are displayed during the data collection.

3. Results and Discussion

HBV (10^6 copies/reaction) can be detected in less than 8 minutes due to the high thermal conductivity of silicon based PCR chips and the small reaction volume of samples. The heating rate of 20°/sec and the cooling rate of 10°/sec have been attained. The volume of 1 uL has been used. Such small volume for the reaction reduces the cost of reagents significantly. The CV (coefficient of variance) of melting temperatures after PCR amplification is less than 0.5% using a home-made SYBR Green I dye-based HBV assay kit (Table 1).

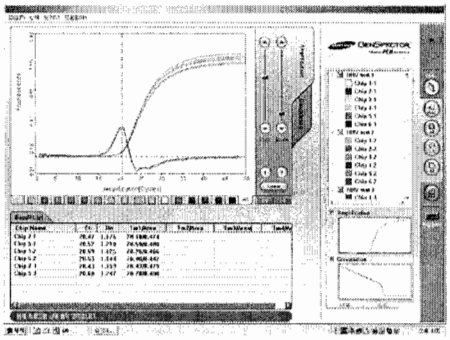

Figure 2. A GenSpector® Micro PCR software.

Table 1. The specifications of GenSpector® Micro PCR system.

Specifications	
Time to Detection (HBV Assay)	< 8 min
Reaction Volume	1 uL
Temperature Accuracy	< ± 0.5 .
Heating Rate	> 20 ./sec
Cooling Rate	> 10 ./sec
Melting Temperature (%CV)	< 0.5 %
Optical Detection	SYBR Green I

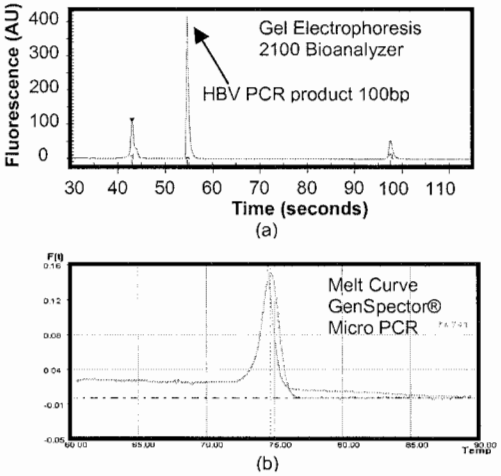

Figure 3. A gel electrophoresis result (a), showing good match to the graph of melt curve (b).

Fig. 3 shows a gel electrophoresis result, showing a good match to the graph of melt curve. The micro PCR system has shown excellent clinical diagnostic results for HBV DNA: 93.8% in sensitivity, 96.4% in specificity, 95.1% in accuracy and 91.7% in reproducibility as shown in Fig. 4 and Table 2.

4. Conclusion

In conclusion, a rapid micro PCR system has shown excellent clinical diagnostic results for HBV DNA detection. Also, the system enables one or more operators to apply for qualitative or quantitative PCR. Thus, the GenSpector® Micro PCR system will have various applications in nucleic acid point-of-care-tests.

References

[1] http://www.GenSpector.com
[2] Dae Sung Yoon, et al, J. Micromech. Microeng. 12 (2002) 813–823.

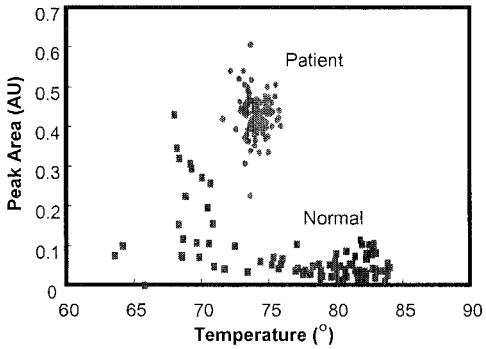

Figure 4. Clinical diagnostic results for HBV DNA detection were plotted as peak area vs. melt temp.

Table 2. Clinical diagnostic result for HBV DNA detection.

	Patient (112)	*Normal (112)*
Positive	TP: 105 (93.8%)	FP: 4
Negative	FN: 7	TN: 108

Where, Sensitivity = TP / (TP+FN),
Specificity = TN / (TN + FP),
Accuracy = (TP+TN)/(TP+FN+FP+TN)
TP: True Positive,
TN: True Negative,
FP: False Positive,
FN: False Negative