Rapid MRSA gene detection device for bedside monitoring

○Kazuya Takahashi[1], Masato Saito[1], Yukihiro Akeda[2], Kazunori Tomono[3], Eiichi Tamiya[1]

1.Department of Applied Physics, Graduate School of Engineering, Osaka University

2. Research Institute for Microbial Diseases, Osaka University

3. Division of Infection Control and Prevention, Graduate School of Medicine, Osaka University

E-mail: saitomasato@ap.eng.osaka-u.ac.jp

【 **Introduction** 】:Avoiding opportunistic infection caused by drug resistant bacteria (i.e., MRSA (Methicillin-resistant *Staphylococcus aureus*)) is crucial especially for weak people in the hospital. MRSA has mecA gene that is the cause of its drug resistance. PCR based genetic test is a gold standard for gene detection and identification. POCT (Point-of-Care Testing) PCR device can be utilized for detection of various inspection targets in many cases such as medicine and food safety. A variety of PCR methods, for example conventional PCR using thermal cycler, flow PCR and convective PCR utilizing thermal convection(*SCIENCE* 298, 793, 2002), have previously been developed. However, these conventional PCR methods require complicated sample preparations, trained users and large apparatus for on-site inspection. Accordingly, PCR system had been developed in our laboratory, which applied thermal convection and centrifugal force, aimed at expediting and simplifying PCR process and downsizing the device. An automated dispensing system had been previously developed, using capillary and centrifugal force to dispense specified amounts of reagents into the micro channel. With these techniques, PCR can be conducted without well-trained users and large apparatus and be applied to on-site diagnosis.

In this report, optimization of MRSA genomic DNA detection was achieved and calibration curve using for various concentration of MRSA genomic DNA was derived. The device has been improved for multi-targeting and bedside inspection. The improved device can be tested for rapid and simultaneous detection of drug resistant bacteria like MRSA.

【**Experiment**】:Rotation device with 8 heaters was fabricated. The temperatures of high(95℃) and low(60℃) heaters were controlled independently. The stage with chips and heaters was rotated by motor, inducing centrifugal force and thermal convection PCR. The PCR solution contains MRSA genomic DNA and mecA primer that was filled up into micro fluidic channel and the mecA amplification was observed at the endpoint based on the fluorescence intensity of Taqman probe. Using 0.94ng/channel MRSA genomic DNA, PCR conditions were optimized by varying the relative gravitational acceleration(3~11G), heater temperature(65.1~75.1℃) and PCR reaction time(5~20min).The experiment was conducted at optimized conditions to create the calibration curve for various concentration of MRSA genomic DNA. An automated dispensing device for bedside diagnosis was designed.

【**Result＆Discussion**】:The temperature on the heaters was checked that these can be kept at 95 and 60℃ that is needed for conducting PCR by using thermo seal. Varying the relative gravitational acceleration, PCR reaction time and temperature of the heater, the observed maximum fluorescence intensity of mecA amplification was at 7G, 15min and 67.1℃, respectively. The gel electrophoresis results confirmed the successful amplifications of the 214 bp fragment of mecA. The detection limit was 94fg/channel MRSA genomic DNA in 15 minutes. This result indicates that multi targeting can be done using the improved 8ch device. In extension, the detection of MRSA using the automated dispensing system is being developed.

© 2015年 応用物理学会