Supplementary Information

Rapid detection for primary screening of Influenza A virus: Microfluidic RT-PCR chip and electrochemical DNA sensor

Keiichiro Yamanaka,a Masato Saito,a Kenji Kondoh,a Mohammad Mosharraf Hossain,a Ritsuko Koketsu,b Tadahiro Sasaki,b Naoki Nagatani,c Kazuyoshi Ikuta,b and Eiichi Tamiya*a

aDepartment of Applied Physics, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan. Tel (+81) 6 6879 4087; Fax (+81) 6 6879 7840; E-mail:tamiya@ap.eng.osaka-u.ac.jp
bDepartment of Virology, Research Institute for Microbial Diseases (BIKEN), Osaka University, 3-1 Yamadaoka, Suita, Osaka 565-0871, Japan.
cDepartment of Applied Chemistry, Faculty of Engineering, Okayama University of Science, 1-1 Ridai-cho, Kita-ku, Okayama 700-0005, Japan.
The influence of nonspecific binding of MB and non-target dsDNA

Methylene blue can bind with the non-target dsDNA molecules. However, the amount of non-target dsDNA is very small compared to the amplified DNA by PCR, so it is negligible. PCR is an exponential amplification of a DNA target sequence depending on the population of initial quantity. Therefore, any dsDNA without target sequences, such as human genomic DNA from the real sample, is negligible compared to the PCR amplified product by the usual detection methods such as the fluorescent observation and/or the electrophoresis. In order to detect the significant decrease of the electrochemical signal derived from MB-dsDNA binding, the obvious amplification such as the clearly bands in the gel electrophoresis, are required. In fact, the peak current values of 536 copies/μL of the template RNA added samples (not amplified) were almost the same as that of the samples using water as template.

Figure S1 shows RT-PCR of negative controls using the human genomic DNA for the electrochemical detection. After RT-PCR, no band was observed by the electrophoresis (Fig.S1A), and the significant decrease of the peak current was not detected in comparison with the case of using water (Fig. S1B). Additionally, RT-PCR using the template solution of influenza A virus RNA mixed with the human genomic DNA was carried out, as shown in Fig. S2. The RT-PCR solution was contained 5360 copies/μL (41.23 fg/μL) of Influenza RNA and 400 pg/μL the human genomic DNA. As a result, the significant decrease in the peak current of the only amplified sample could be measured. Therefore, a large amount of non-target dsDNA compared with that of target RNA is not influenced for the electrochemical detection of RT-PCR. As described above, using the water for non-template control is not to be a problem for the microfluidic RT-PCR and the electrochemical measurements and it is considered that dsDNA from the real sample will cause any problem.

Fig. S1. RT-PCR of negative controls using human genomic DNA. The genomic DNA of 0.4, 4, 40, 400 pg/μL and water were added to the RT-PCR solutions. (A) The photograph of electrophoresis. (B) The electrochemical measurements (SWV). Theses peak currents were -11.28 μA (water), -11.62 μA (0.4 pg), -11.01 μA (4 pg), -11.65 μA (40 pg), -11.07 μA (400 pg).
The influence of RT-PCR solution for the accurate electrochemical detection of MB

It was confirmed that there are no other electro-active compounds in the RT-PCR solution that can give a signal at the peak potential of MB. The electrochemical signals of RT-PCR samples with and without methylene blue were measured as shown in Fig. S3. The reduction peak signal of methylene blue was observed around at –0.38 V. In the case of after RT-PCR solution without methylene blue, the peak signal was not detected around –0.38 V (Fig. S3B). Therefore, the compounds in RT-PCR solution were not having the electrochemical activity in this potential region, and there are no other overlapping signals in the results of methylene blue measurements.
Fig. S3. The electrochemical measurements of after RT-PCR solutions with and without methylene blue. (A) the photograph of electrophoresis. (B) the SW Voltammograms of RT-PCR samples without methylene blue. (C) the electrochemical measurements compared with the methylene blue contained samples.
NC: negative amplification. PC: positive amplification