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Short Communication

Nucleic acid purification from dried blood spot on FTA Elute Card provides template for polymerase chain reaction for highly sensitive *Plasmodium* detection☆

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ABSTRACT

Polymerase chain reaction (PCR) is an essential diagnostic method for highly sensitive detection of *Plasmodium*-infected erythrocytes in patients with malaria. This study compared the performance of filter papers used for the preparation of dried blood spots (DBS) in detecting *Plasmodium* by PCR. Whole blood spiked with *P. falciparum*-infected erythrocytes to obtain samples with various levels of parasitemia were applied to Whatman 3MM Chr papers, FTA Cards, or FTA Elute Cards to prepare the DBS. DNA was purified from the DBS using a DNA purification kit and used as the template for nested PCR. In probit analysis, the estimated limit of detection (LoD) was 5.5 parasites/μL blood for Whatman 3MM Chr papers and FTA Cards and 1.6 parasites/μL blood for the FTA Elute Card. This result suggested that the DBS prepared on an FTA Elute Card yield the best template DNA for subsequent high-sensitivity PCR-based detection of *P. falciparum*-infected erythrocytes. This finding can help improve the accuracy of malarial diagnostic tests.

1. Introduction

Individuals with sub-microscopic, low-density *Plasmodium* infections are considered carriers contributing to ongoing transmission of malaria. Therefore, surveillance of these infections is important for malarial control [1]. Polymerase chain reaction (PCR) is an essential diagnostic method for highly sensitive detection of *Plasmodium*-infected erythrocytes in patients with malaria. Furthermore, PCR is used for the identification of different *Plasmodium* species [2]. However, PCR-based methods cannot be routinely used in the field as it requires equipment such as a centrifuge for nucleic acid purification and a thermal cycler. Thus, for collection and preservation of samples, as well as to transport the collected samples for PCR analysis in the laboratory, dried blood spots (DBS) on filter papers are used [3]. Efficient extraction of stable, high-quality nucleic acid from DBS is a very important step for reliable and highly sensitive PCR analysis.

Filter papers like thick Whatman filter papers or FTA (Flinder Technology Associates) cards, which were developed for nucleic acid sample preparation, are often used for DBS preparation [4,5]. Nucleic acid purification from DBS is generally performed using commercially available purification kits. Hemoglobin inhibits PCR [6], but hemoglobin from blood samples irreversibly binds to FTA Elute Cards [7]. Hence, the template DNA for PCR can be extracted from DBS in water by heating at 95 °C without using a purification kit. The utility of FTA Elute Cards for diagnosis of malaria has been reported for patients with high parasitemia [7,8], but not for those with low parasitemia.

In this study, we compared the performance of Whatman 3MM filter paper, FTA Card, and FTA Elute Card for preparing DBS for subsequent PCR-based detection of *Plasmodium falciparum* in whole blood spiked with infected erythrocytes. DNA was purified from the DBS using a DNA purification kit and then used as the template for nested PCR with primers specific for *P. falciparum 18S rRNA* gene. Each filter paper was

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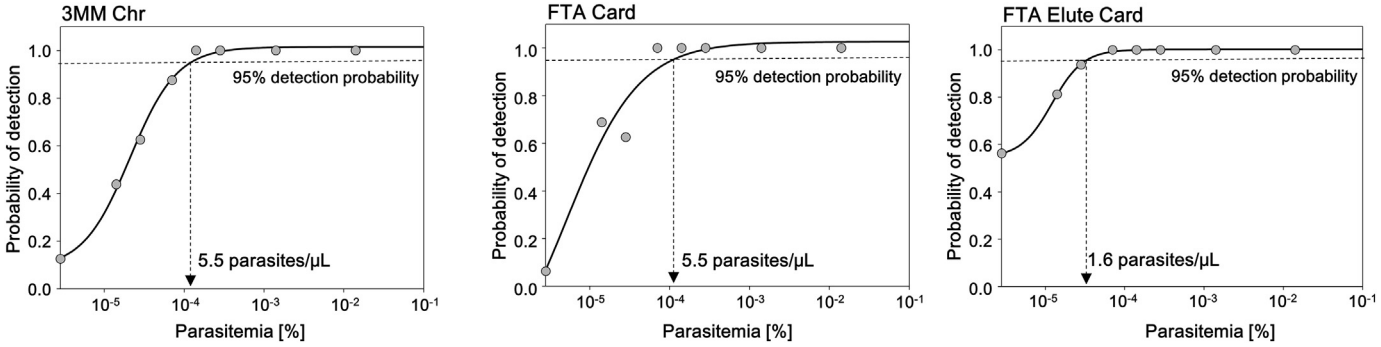

Fig. 1. LoD of nested PCR for DBS on 3MM Chr paper, FTA Card, and FTA Elute Card.

evaluated for appropriateness of the DNA recovered from DBS by estimating the limit of detection (LoD) of the nested PCR. DNA purified from DBS on FTA Elute Card was found to be the most appropriate as the template for highly sensitive PCR.

2. Methods and methods

2.1. Filter papers

Thick filter paper for blotting (Whatman 3MM Chr papers), Whatman FTA MicroCards (CAT No. WB120210), and Whatman FTA Elute Micro Cards (CAT No. WB120410) were purchased from GE Healthcare Bio-Science Corp. (Pittsburgh, PA, USA).

2.2. Preparation of whole blood spiked with cultured parasite-infected erythrocytes

P. falciparum was cultured essentially as previously described [9]. Briefly, *P. falciparum* 3D7 strain was cultured in RPMI 1640 medium (Nacalai Tesque, Inc., Tokyo, Japan) supplemented with 50 μg/mL gentamycin (SIGMA-Aldrich Co., St. Louis, MO, USA) and 10% O^+ human serum at a hematocrit of 3%. The cultured parasites were added to human erythrocytes (blood group O^+) that were obtained [1–13] from the Japanese Red Cross Society (Tokyo, Japan) and this suspension was incubated at 37 °C under 5% CO_2 and 90% N_2 gas to obtain parasite-infected erythrocytes. We synchronized the parasites at the ring stage using 5% D-sorbitol and harvested highly synchronized ring-stage parasite-infected erythrocytes (> 95%) [10]. Next, an appropriate volume of the purified parasite-infected erythrocyte suspension was added to whole blood from a healthy volunteer who had never been infected with the parasites to generate a blood sample with 0.14% parasitemia at a hematocrit of 50%. Parasitemia was estimated by thin-smear microscopy with Giemsa staining as described previously [11]. Serial dilution of the 0.14% sample with whole blood yielded prepared blood samples with parasitemia ranging from 0.0000028% to 0.014% and a hematocrit of 50%. For preparation of DBS, 20 μL of the prepared blood samples was applied to each of the three tested filter papers and the papers were dried for > 3 h. These filter papers were stored protected from light at 23 °C for > 20 days. They were then used for DNA extraction.

2.3. Sample preparation and PCR

DNA was extracted from DBS containing 20 μL of the prepared blood on Whatman 3MM Chr papers, FTA Cards, or FTA Elute Cards using the QIAamp DNA Micro Kit (Qiagen, Venlo, Netherlands) according to the method described in the manual. DNA was eluted in 20 μL of water. For FTA Elute Card, DBS was rinsed thrice with 500 μL of water by pulse vortexing. Next, 30 μL of water was added to the DBS and the card was incubated at 95 °C for 30 min. Nested PCR amplification of the *P. falciparum 18S rRNA* gene was performed as previously described [4]. Positive and negative PCR results were determined by examining the gel image with the naked eyes.

2.4. Statistical analysis

Data analysis was performed with SigmaPlot Version 13 (Systat Software, Inc., San Jose, CA, USA). The LoD of nested PCR assays, *i.e.*, the concentration at which a sample is detected as positive with 95% confidence, was calculated by probit analysis of the results from the serial dilution.

3. Results and discussion

Probit analysis was used to determine the LoD based on the results of nested PCR analysis of the prepared blood samples. To compare the performance of the filter papers, each sample was applied to Whatman 3MM Chr papers, FTA Cards, and FTA Elute Cards and then dried. DNA was purified from DBS using a DNA purification kit, and a 1 μL aliquot was used as template in a 12.5 μL PCR mixture. The analytical sensitivity of nested PCR was calculated using an eight-point standard dilution series (0.014%, 0.0014%, 0.00028%, 0.00014%, 0.00070%, 0.000028%, 0.000014%, 0.0000028% parasitemia). The experiments were performed in sixteen replicates. A graphical illustration of the probit analysis is presented in Fig. 1.

The estimated LoD was 0.00011% parasitemia (5.5 parasites/μL blood, $R = 0.9981$) for 3MM Chr paper, 0.00011% parasitemia (5.5 parasites/μL blood, $R = 0.9746$) for the FTA Card, and 0.000031% parasitemia (1.6 parasites/μL blood, $R = 0.9998$) for the FTA Elute Card, demonstrating that the FTA Elute Card provided the most appropriate template for sensitive PCR. By contrast, when DNA was extracted from DBS on the FTA Elute Card by heating the DBS in water, as described in the manual, the probability of detection at 0.014% parasitemia was 0.81 (13 positive results/16 PCR replicates) and at 0.0014% parasitemia was 0.063 (1 positive result/16 PCR replicates), indicating that these extracted samples served as low-quality templates for PCR. These findings show that use of a DNA purification kit is essential for extracting high-quality DNA suitable for highly sensitive PCR from DBS on the FTA Elute Card.

Highly sensitive detection of *Plasmodium* by nucleic acid amplification methods, including PCR, is crucial for elimination of malaria by blocking transmission from asymptomatic infected individuals with very low parasitemia [12]. In general, on-field DNA purification and PCR are difficult to perform in endemic countries; hence, blood is collected as DBS and transported and analyzed in equipped laboratories often in developed countries. In this context, the filter paper used for preparing the DBS is a critical factor determining the accuracy of the diagnostic test. Although some laboratories have reported LoD ranging from 0.1 to 10 parasites/μL in blood for nested PCR [13], the filter papers and/or methods used for estimation of the LoD are not

standardized. To our knowledge, this is the first report examining the performance of various filter papers in PCR-based analysis of whole blood spiked with parasite-infected erythrocytes at 95% detection probability.

The utility of FTA Elute Cards for *Plasmodium* detection *via* DNA extraction by heating has been reported, but the blood samples used showed relatively high parasitemia (0.3% to 0.6%) [8]. In the present study, good PCR template DNA could be purified from DBS on FTA Elute Cards using a commercially available DNA purification kit that is often used in malarial research. Hemoglobin strongly inhibits PCR [6]. Since hemoglobin binds strongly to the FTA Elute Card, it is not extracted even if the DBS were incubated with proteinase K supplied in the DNA purification kits. This feature of the FTA Elute Card might be responsible for its superior DNA purification ability compared with the other tested filter papers. Effective purification of DNA from DBS is very important for PCR. Although FTA Elute Card is more expensive than Whatman 3MM Chr papers or FTA Card, FTA Elute Card should be used for preparation of DBS for PCR with high sensitivity.

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