Brief Report

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# Short Report. DNA Contamination during Extraction of Dried Blood Spots from Whatman Filter Paper

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**Abstract:** Dried blood spot (DBS) testing on confetti is one of the most common methods used to collect and extract DNA from field samples for molecular epidemiology of malaria studies. Here, we investigated whether ethanol or other solutions used to clean scissors are a source of sample contamination. DBS were prepared from 3 drops of blood from two *Plasmodium falciparum* cultures spotted on confetti, and 3 solutions (water, ethanol and DNase) were used to clean scissors between spots during DNA extraction. For each cleaning solution, two blank confetti were used as negative controls. Samples were analyzed by PCR-based genotyping of merozoite surface proteins 1 & 2 (*msp-1* and *msp*-2), and *P. falciparum* chloroquine resistance transporter *(Pfcrt)*. A total of 15 samples were analyzed. Based on *msp*-1 and *msp*-2 amplification, *P. falciparum* was detected on blank confetti when scissors were washed with ethanol. Based on *Pfcrt* amplification, DNA of *P. falciparum* was detected on blank confetti when scissors were cleaned with ethanol or water. No *P. falciparum* genes were detected on blank confetti when scissors were cleaned with DNase. Scissors cleaned with DNase prevented cross-contamination between samples during processing of dried blood spots, whereas ethanol (which is commonly used) fails to avert cross-contamination.

**Keywords:** DNA extraction; Scissors; DBS; DNase

## 1. Introduction

Malaria is a serious global health problem causing over 600,000 deaths in 2021 [1]. Correct diagnosis is necessary for prevention, control, and suitable treatment of malaria, and commonly performed by microscopy, rapid diagnostic tests (RDTs), and molecular methods [2]. Success of a molecular method for malaria diagnosis depends critically on the quality of genomic DNA being used for examination, which in turn depends on accuracy of the employed isolation procedure.

Multitudes of different DNA extraction methods have been published [3] since the first DNA isolation in 1869 [4], and each method must overcome specific extraction problems such as DNA shearing, background contamination, low purity, and low yield. Of these, dried blood spot (DBS) is one of the most common methods used to collect and extract DNA in field studies of malaria molecular epidemiology, where transport and storage conditions are often suboptimal. This technique is minimally invasive (requiring only microliters of typically peripheral blood), inexpensive, and easy to multiplex and automate [5]. DBS samples are compatible with many bioanalytical methods, among them chromatography, mass spectrometry, DNA, and immunoassays. However, DNA extraction is particularly challenging in resource-limited regions, where scissors are used to cut filter papers, possibly introducing contamination between samples due to poor scissor cleaning.

Many DBS methods use 70% ethanol to clean the scissors, but it is unknown whether ethanol or other cleaning solutions may be a source of sample contamination. We drafted this letter to shed light on concerns regarding contamination of samples extracted by scissors which may impact interpretation of molecular epidemiology results.

## 2. Methods

### *2.1. DNA Extraction from DBS*

DBS were prepared from 3 drops of blood from the same culture, spotted on Whatman FTA cards (Whatman Inc., Brentford, UK), then dried and stored for 24 hours at room temperature (**Figure 1**). DBS on cards were cut into small pieces with scissors and transferred into 1.5-mL microtubes. Parasite DNA was extracted using QIAamp DNA blood mini kits (Qiagen, Valencia, CA USA) according to the manufacturer's instructions and final eluted volume was 30 mL. DNA samples were stored at −20°C until use. The concentration of DNA was achieved using a NanoDrop2000.

DBS were prepared from 3 drops of blood from two *Plasmodium falciparum* cultures (20-0119-0 and 20-2091-0 isolates) spotted on filter paper (**Figure 2**). All samples were dried for 24 hours at room temperature. We examined three solutions (water, ethanol, and DNase) to clean scissors between spots during DNA extraction. For each cleaning solution, two blank confetti were used as negative controls. DBS were cut into small pieces with scissors and transferred into 1.5-mL microtubes. Residual water (RW), ethanol (RE) and DNase (RD) after scissor washing were also amplified.

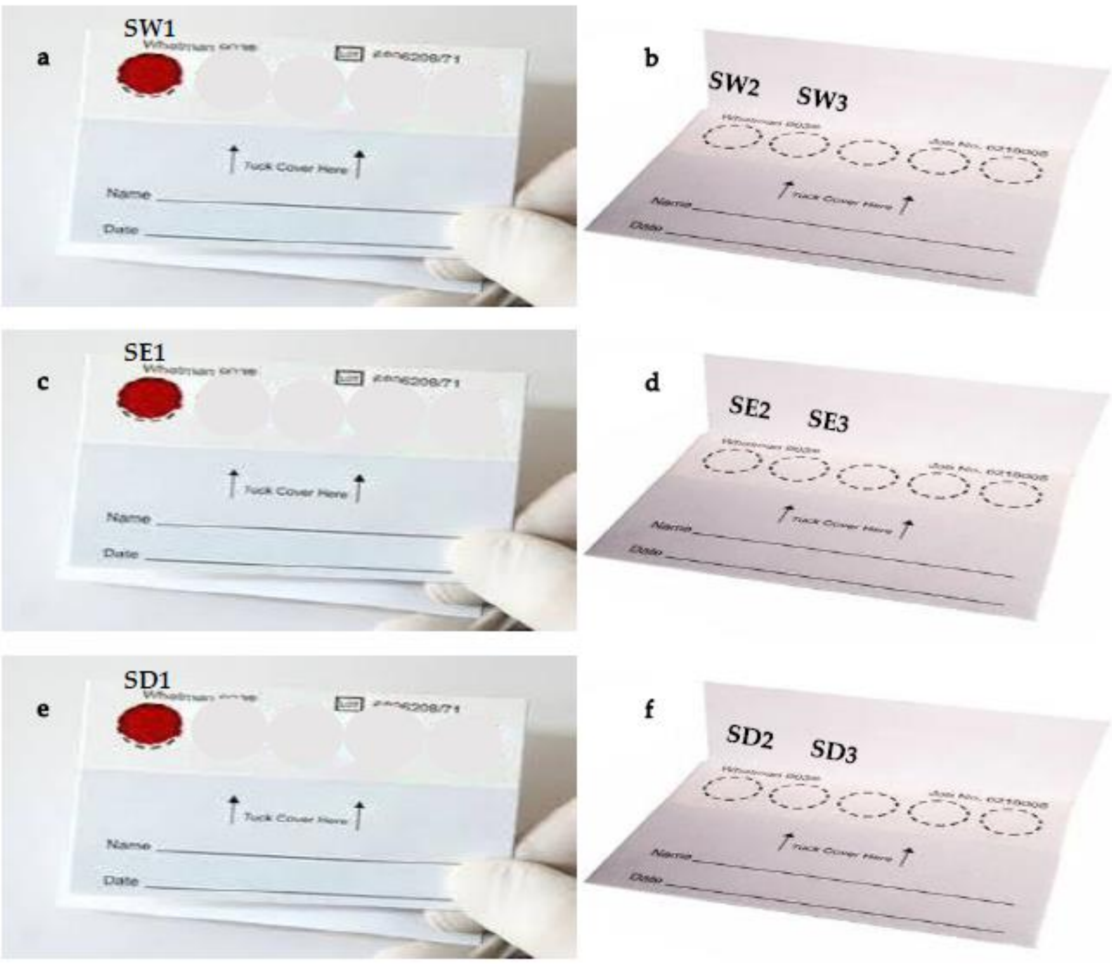


**Figure 1. Dried blood spots prepared using scissors washed by water, ethanol, and DNase.** (a,b) SW1 is the reference with blood and SW2 and SW3 are blank confetti catted after washing scissors with water the first and second time, respectively. (c-d) SE1 is the reference with blood and SE2 and SE3 are blank confetti catted after washing scissors with ethanol the first and second time, respectively. (e-f) SD1 is the reference with blood and SD2 and SD3 are blank confetti catted after washing scissor with DNase the first and second time, respectively.

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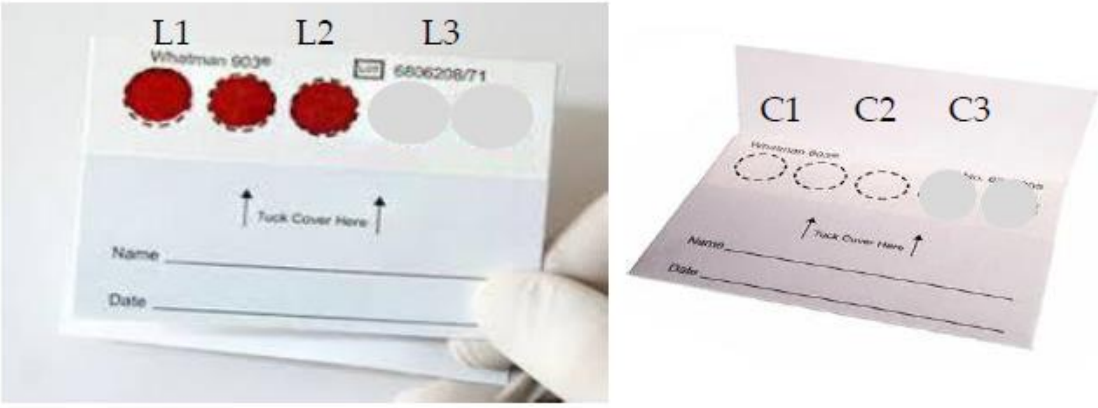


**Figure 2.** Dried Blood spot: L1 and L2 are the reference with blood and C1 and C2 are blank confetti catted after washing scissors with ethanol the first time, respectively; L3 is the reference with blood and C3 is blank confetti catted after washing scissors with DNase the first time.

### *2.2. PCR Assay of DBS*

Parasite-infected and negative control samples were analyzed by PCR-based genotyping of *P. falciparum* merozoite surface proteins 1 & 2 (*msp-1* and *msp*-2), and *P. falciparum* chloroquine resistance transporter *(Pfcrt)* genes. A total of 15 samples were analyzed. Based on *msp*1 and *msp*2 amplification (3 for each cleaning solution, n=9), *P. falciparum* was detected on blank confetti when scissors were washed with ethanol. Based on *Pfcrt* amplification (2 for each cleaning solution, n=6), DNA of *P. falciparum* was detected on blank confetti when scissors were washed with ethanol or water. Genotyping using amplification of *msp-1* and *msp-2* markers were performed on paired samples obtained.

PCR primers sequences and cycles have been previously described [6]. Gels were stained with ethidium bromide and visualized under UV illumination. Band sizes of PCR products across the two markers were measured visually and compared. PCR products visualized on 1.5% agarose gel in 1×Tris-acetate-EDTA (TAE) and stained with ethidium bromide (Sigma-Aldrich, Sydney, NSW, Australia). Length differences were determined using a 100 bp molecular weight ladder (Promega). Electrophoresis conditions were 100 mV for 30 min. Allele-specific positive controls and DNA-free negative controls were included in each round of reactions. Primers for *msp-1* and *msp-2* were synthesized by Integrated DNA Technologies, Coralville, IA, USA.

## 3. Results and Discussion

Results of the nested PCR revealed that no bands were detected in samples washed with DNase. Schematic representation of agarose gel electrophoresis of nested PCR products from SW1, SE1 and SD1 show the presence of bands at 565 bp and 534 bp in *msp1* (**Figure 3**) and *msp-2* (**Figure 4**) respectively. In the nested PCR assay using *msp-1* and *msp-2*, no band was detected in SW1, SW2, SE2, SD1, SD2, RE and RD tested. We detected bands in SE2 in *msp-1* and SE1 and SE2 in *msp-2*, respectively. Only blank confetti catted by scissors washed with ethanol presented bands, detected in C1 and C2 on agarose gel electrophoresis of digestion PCR products, but no band was detected with C3 (**Figure 5**).

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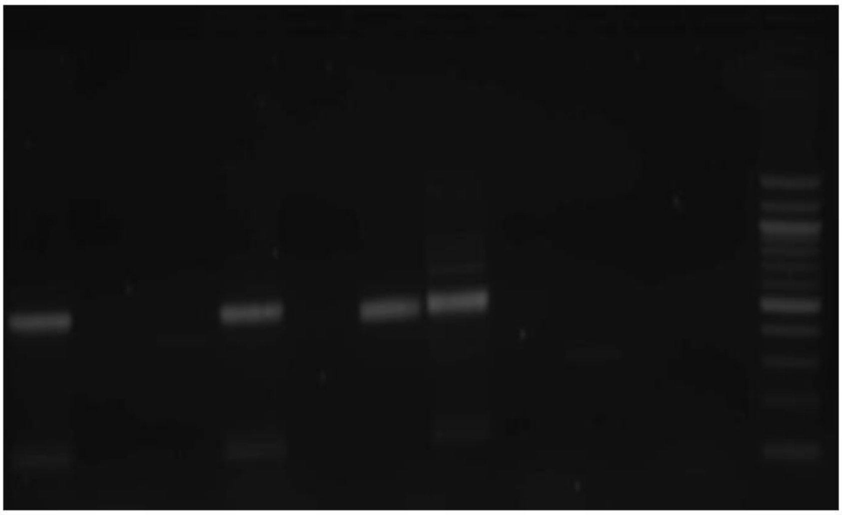

**Figure 3.** *msp-1* products from the PCR 2 run on a 1.5% agarose gel. MP, 100 bp molecular weight ladder (Sigma P1473). DBS catted by scissors washed with water (SW1 as reference, SW2 and SW3 from blank confetti); DBS catted by scissors washed with ethanol (SE1, SE2 and SE3) and DBS catted by scissors washed with DNase (SD1, SD2 and SD3). RW, RE and RD represent residual water, ethanol, and DNase, respectively.

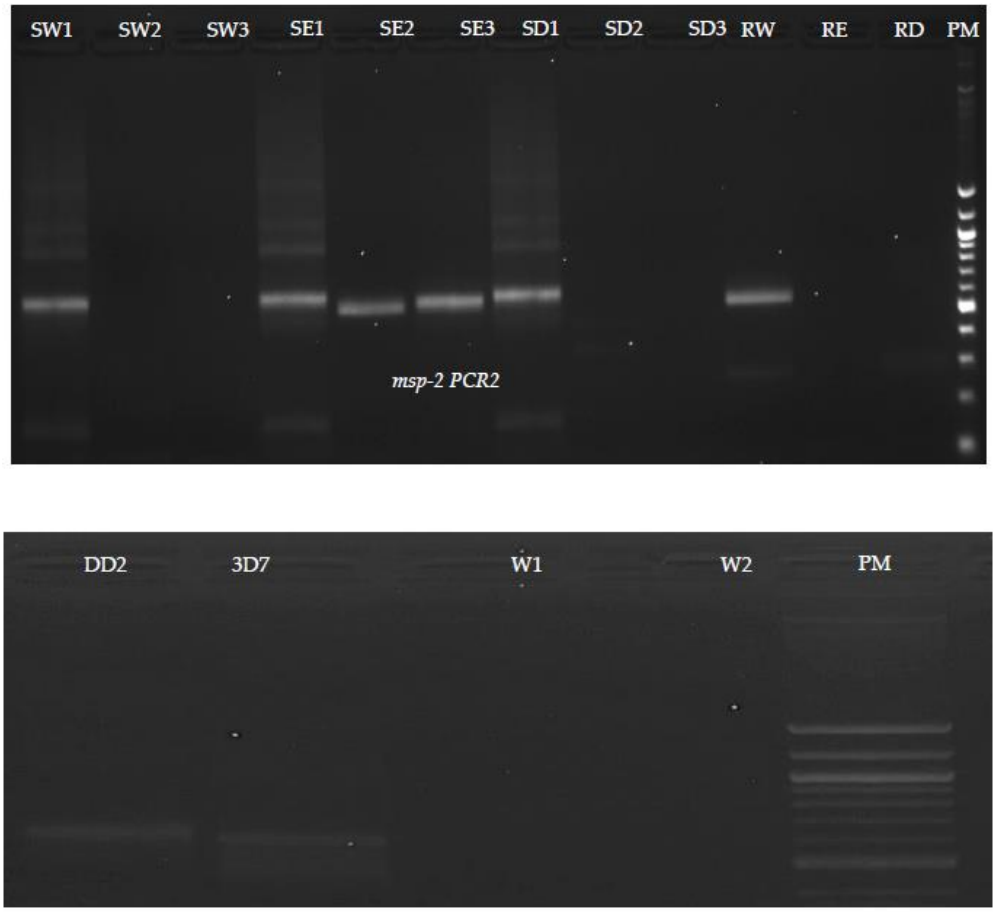


**Figure 4.** *msp-2* products from the PCR 2 run on a 1.5% agarose gel. MP, 100 bp molecular weight ladder (Sigma P1473). DBS catted by scissors washed with water (SW1 as reference, SW2 and SW3 from blank confetti); DBS catted by scissors washed with ethanol (SE1 as reference, SE2 and SE3) and DBS catted by scissors washed with DNase (SD1 as reference, SD2 and SD3). RW, RE and RD

represent residual water, ethanol, and DNase, respectively. DD2 and 3D7 are positive controls and water (W1 and W2) is negative control; PM is molecular marker.

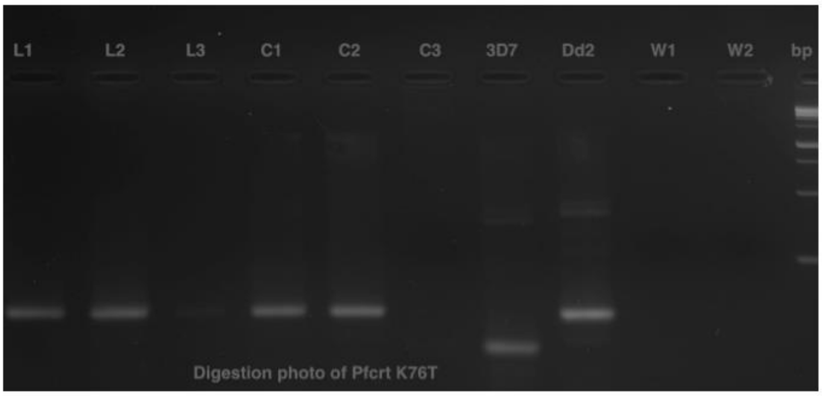


**Figure 5.** Illustration of the Figure 2 experience. *Pfcrt* products from the digestion run on a 1.5% agarose gel. 100 bp molecular weight ladder (Sigma P1473). DBS catted by scissors washed with ethanol (L1, L2 as reference, C1 and C2) and DBS catted by scissors washed with DNase (L3 as reference, C3). W1, W2, 3D7 and Dd2 represent controls, respectively.

Ethanol is a non-additive precipitant fixative that shifts isoelectric points of proteins less than other fixatives [7]. It fixes proteins by dehydration and precipitation, the degree of which being dependent on the amount of water present and the solubility of materials in the mixture. The basic aims of fixation are to preserve the tissue nearest to its living state, and to prevent any change in shape and size of tissue at the time of processing. This could possibly explain how pieces of blood-stained confetti attached to scissors after being dipped in ethanol. In addition, we observed one band from residual of water (RW) in *msp*-2. Water is by far the most studied chemical compound [8] and called the universal solvent since it can dissolve more substances than any other liquid [9]. After dipping in water, particles of confetti on scissors detached themselves and remained at the bottom of the water. DNase is an endonuclease that digests single and double-stranded DNA; it hydrolyzes phosphodiester bonds producing mono- and oligodeoxyribonucleotides with 5'-phosphate and 3'OH groups. The enzyme activity is strictly dependent on Ca2+ and is activated by Mg2+ or Mn2+ ions: 1) in the presence of Mg2+, DNase cleaves each strand of dsDNA independently in a statistically random fashion [10]; 2) In the presence of Mn 2+, the enzyme cleaves both. DNA strands at approximately the same site, producing DNA fragments with blunt ends or with one or two nucleotide overhangs [10]. We did not detect any band with *msp-1* and *msp-2* in scissors washed with DNase.

## 4. Conclusions

Scissors cleaned with DNase prevented cross-contamination between samples during processing of dried blood spots. Ethanol cleaning of scissors, which is commonly used, failed to avert cross-contamination. Sample cross-contamination can reduce diagnosis accuracy; we recommend DNase wash as the standard cleaning solution to improve DNA extraction.

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**Conflicts of interest:** The authors declare no competing interest.

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