Short Communication

Evaluation of contamination associated with current blood spot technology for determining the fatty acid status of individuals

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A newly developed method for determining fatty acid status in samples of whole blood using collection papers was evaluated. Fresh blood was analyzed either directly or after being applied to a strip of blood collection paper. When compared with fresh blood, spotted blood samples exhibited a higher percentage of saturated fatty acids and lower percentage of polyunsaturated fatty acids, especially when the volume of blood spot was low, suggesting the presence of contaminants in the paper. This was confirmed by gas chromatography analysis of blank collection papers which produced peaks which corresponded with saturated fatty acids. Further studies revealed that latex gloves, aluminium bags and polyethylene bags also have the potential to contaminate blood spot samples during sample collection, processing, and storage. The choice of collection paper, gloves, and storage bags which contain the minimum amount of contaminants is therefore essential to reduce the impact of external contaminants on the blood spot fatty acid analysis results.

Practical applications: This research shows that the choice of collection paper, gloves, and storage bags which contain the minimum amount of contaminants is essential to reduce the impact of external contaminants on the blood spot fatty acid analysis results. Moreover, this work shows the potential of using Whatman ion exchange paper in clinical trials that are aimed at measuring the fatty acids composition in dried blood spots, to provide more accurate results.

Keywords: Blood spot / Collection paper / Contaminant / Fatty acid composition

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1 Introduction

The positive effect of dietary omega-3 long chain polyunsaturated fatty acids (LCPUFA) in maintaining good health has become an increasingly important area of research in recent years. Epidemiological studies and clinical trials have demonstrated that increased intake of n-3 LCPUFA, especially eicosapentaenoic acid (20:5 n-3) and docosahexaenoic acid (22:6 n-3), are associated with prevention of preterm birth [1], primary, and secondary protection against cardiovascular disease [2] and anti-inflammatory effects [3].

The fatty acids status in blood is a useful marker for evaluating cardiovascular risk in human populations and identifying those individuals who would benefit from n-3 LCPUFA supplementation [4]. However, the conventional assay of fatty acids in blood involves venous blood collection and an expensive, time consuming multi-step process that limits its usefulness as a screening tool. Recently, a simplified method for the determination of blood fatty acids has been reported [5]. In this method, a blood spot from a finger prick is collected on a piece of chromatography paper, all lipid classes in the blood spot are transmethylated directly and the

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Abbreviations: FAME, fatty acid methyl esters; **FID,** flame ionisation detector; **GC,** gas chromatography; **LCPUFA,** long chain polyunsaturated fatty acids

fatty acid methyl esters analyzed by gas chromatography (GC) to evaluate fatty acids status. This blood spot method is quicker and cheaper than the conventional method, thus making it ideal for large clinical trials and as a diagnostic test. However, attempts in our laboratory to validate this methodology found that contaminants from certain types of collection papers may reduce the accuracy of fatty acid analysis results of blood spot samples [6].

The objective of this study was to evaluate the level of contaminants in different collection papers and to develop a method which would minimize the contaminant problem.

2 Materials and methods

2.1 Blood spot collection papers

Blood spot collection papers from eight sources were tested, including five commercial blood spot collection products: Fluka blood collection kit (Sigma–Aldrich, Buchs, Switzerland), Hemaspot-80 blood collection paper (Spot on science, Austin, USA), Whatman 903 specimen collection card (three batches, all from same manufacturer) (Whatman, Buckingham, UK), and three chromatography papers: Whatman 3MM chromatography paper (46 × 57 cm), Whatman ion exchange chromatography paper (SG81, 46 × 57 cm), and Whatman glass microfiber filter (GF/B 47 mm). The Fluka blood collection kit is a commercial product, which is specifically marketed for the measurement of blood n-3 fatty acid status. The hemaspot-80 plate kit is another commercial blood collection tool which is used for the screening of several analytes, including fatty acids, proteins, and DNA/RNA, in humans. The Whatman 903 specimen collection paper is a blood collection paper routinely used for the diagnostic screening test for various metabolic disorders performed on every infant in Western countries (Guthrie test). The Whatman 3 MM chromatography paper has been used as a blood collection paper in clinical trials for the identification of blood disorders [7], and for the measurement of blood cholesterol and triglycerides levels [8]. Whatman ion exchange chromatography paper and Whatman glass microfiber filter paper were chosen to compare with other blood collection papers, since we had previously shown that it has superior dried blood spot LCPUFA protective properties to other collection papers [9].

2.2 Sample preparation

Ethical approval for the collection of blood and body fluids for this study was obtained from The University of Adelaide Human Research Ethics Committee. The volunteer was fully informed of the nature of the study and provided written informed consent prior to blood collection. For the studies reported in this manuscript, approximately 5 mL of blood was collected from one healthy volunteer (male, aged 30) through the antecubital vein to a 6 mL collection tube containing heparin as anticoagulant.

Since we had previously shown that the process of air-drying itself can affect the fatty acid composition of blood spots [9], whole blood samples spotted on to paper were analyzed prior to drying. In order to directly evaluate the levels of fatty acids in whole blood, triplicate samples of 25 μL of fresh whole blood were directly transmethylated with 2 mL of 1% (v/v) H_2SO_4 (18M AR grade, BDH, Sussex, UK) in anhydrous methanol in a 5 mL sealed vial (Wheaton, Millville, USA) heated at 70°C for 3 h. The resultant fatty acid methyl esters (FAME) were extracted into heptane and injected into a GC for analyses according to our previously established method [10]. At the time of blood collection, triplicate aliquots of two volumes of the blood sample (25 μL and 50 μL, respectively) were placed on paper strips (1.5 × 1.5 cm) (Table 1), and processed immediately following the same procedure as for the fresh whole blood. The internal standard methyl heptadecanoate (17:0, Sigma–Aldrich, ≥99%) was added to the fresh whole blood in order to allow for the quantitation of fatty acid concentrations in the samples.

The eight types (or batches) of blank collection paper strips of an equivalent area (1.5 × 1.5 cm) without blood were spiked with internal standard methyl heptadecanoate and subjected to the same methylation process as blood spot samples to evaluate the natural amount of contaminants that each type of collection paper released during the methylation process (Table 2). All the samples were processed in triplicate.

To evaluate whether it was possible to wash off contaminants from collection papers, each type (or batch) of blank collection paper strips of the same size (1.5 × 1.5 cm) were soaked in methylating agent at ambient temperature for 1 h, 3 h, and 10 h or at 70°C for 1 h, 2 h, and 3 h (Table 3). After soaking, the collection paper strips were dried at room temperature and processed in fresh methylating agent using the method described above, and analyzed by GC to evaluate the residual amount of contaminants in each type (or batch) of paper. All the samples were processed in triplicate.

To evaluate the potential contaminants from sources other than paper during sample collection, processing, and storage, blank Whatman glass microfiber filter strips (1.5 × 1.5 cm) were wiped on a 10 cm^2 surface area of various materials used in our laboratory including a latex glove, a nitrile glove, a polyethylene (PE) ziplock bag, an aluminium foil ziplock bag, and a cellophane bag (Table 4). The wiped samples of paper were spiked with the internal standard (methyl heptadecanoate) and then methylated using the method described above and analyzed by GC. The contaminant contents of blank glass microfiber filter were subtracted from wiped glass microfiber filters to determine which of the contaminants were derived from the gloves and bags. All the samples were processed in triplicate.

Table 1. Fatty acid composition (%) determined in 25 μL and 50 μL of blood spotted on five types of collection paper compared with fresh blood

Fatty acid	Fresh blood 25 μL		Blood spot Whatman Glass fibre filter		Ion exchange paper		3 MM paper		903 Paper #1		Fluka test kit	
	(μg)*	(%)**	25 μL (%)	50 μL (%)	25 μL (%)	50 μL (%)	25 μL (%)	50 μL (%)	25 μL (%)	50 μL (%)	25 μL (%)	50 μL (%)
16:0	18.7 ± 0.3	22.8 ± 0.4^{a}	22.7 ± 0.3^{a}	22.9 ± 0.3^{a}	22.9 ± 0.2^{a}	23.0 ± 0.2^{a}	23.7 ± 0.1^{b}	23.2 ± 0.1^{a}	25.2 ± 0.5^{c}	24.1 ± 0.2^{b}	24.2 ± 0.4^{b}	23.6 ± 0.2^{ab}
18:0	8.6 ± 0.1	10.5 ± 0.1^{a}	10.6 ± 0.1^{a}	10.7 ± 0.1^{a}	10.6 ± 0.2^{a}	10.5 ± 0.1^{a}	10.8 ± 0.2^{ab}	10.7 ± 0.1^{a}	11.8 ± 0.2^{c}	11.1 ± 0.1^{b}	11.2 ± 0.1^{b}	10.9 ± 0.2^{ab}
18:1n-9	17.4 ± 0.2	21.2 ± 0.2^{a}	21.2 ± 0.1^{a}	21.1 ± 0.2^{a}	21.0 ± 0.2^{a}	21.2 ± 0.2^{a}	21.0 ± 0.1^{ab}	21.3 ± 0.1^{a}	20.2 ± 0.3^{b}	20.2 ± 0.3^{b}	20.7 ± 0.3^{b}	20.9 ± 0.3^{a}
18:2n-6	20.3 ± 0.3	24.8 ± 0.3^{a}	24.7 ± 0.3^{a}	24.6 ± 0.1^{a}	24.8 ± 0.2^{a}	24.7 ± 0.2^{a}	24.2 ± 0.1^{b}	24.5 ± 0.1^{a}	23.5 ± 0.2^{c}	24.0 ± 0.3^{b}	24.0 ± 0.2^{b}	24.4 ± 0.2^{ab}
20:4n-6	7.4 ± 0.04	9.0 ± 0.05^{a}	9.1 ± 0.1^{a}	9.0 ± 0.15^{a}	9.0 ± 0.1^{a}	9.1 ± 0.05^{a}	8.8 ± 0.2^{ab}	8.8 ± 0.1^{a}	8.2 ± 0.2^{c}	8.5 ± 0.1^{b}	8.5 ± 0.1^{b}	8.7 ± 0.2^{ab}
20:5n-3	1.06 ± 0.02	1.30 ± 0.03^{a}	1.30 ± 0.02^{a}	1.27 ± 0.05^{a}	1.28 ± 0.03^{a}	1.28 ± 0.03^{a}	1.22 ± 0.02^{ab}	1.25 ± 0.03^{a}	1.10 ± 0.03^{c}	1.16 ± 0.02^{b}	1.18 ± 0.02^{b}	1.23 ± 0.02^{ab}
22:5n-3	0.98 ± 0.01	1.20 ± 0.02^{a}	1.20 ± 0.03^{a}	1.25 ± 0.02^{a}	1.20 ± 0.01^{a}	1.20 ± 0.05^{a}	1.16 ± 0.03^{ab}	1.18 ± 0.03^{a}	1.10 ± 0.02^{b}	1.16 ± 0.03^{a}	1.15 ± 0.02^{ab}	1.16 ± 0.02^{a}
22:6n-3	3.14 ± 0.02	3.85 ± 0.02^{a}	3.83 ± 0.02^{a}	3.88 ± 0.02^{a}	3.85 ± 0.03^{a}	3.83 ± 0.05^{a}	3.76 ± 0.02^{ab}	3.82 ± 0.02^{a}	3.58 ± 0.03^{c}	3.68 ± 0.02^{bc}	3.68 ± 0.03^{bc}	3.77 ± 0.02^{ab}

*Amount of fatty acids (μg) in 25 μL of fresh whole blood, values represent mean ± SD ($n = 3$).
**Fatty acid composition, values represent mean ± SD ($n = 3$), different superscripts indicate significant difference between groups, $P < 0.05$.

Table 2. Amount of contaminants from different types of collection paper

Contaminants corresponding to fatty acid peaks*	Whatman glass fibre filter	Whatman ion exchange paper	Whatman 3 MM paper	Fluka test kit	Hemaspot-80	Whatman 903 paper #1	Whatman 903 paper #2	Whatman 903 paper #3
16:0	0.1 ± 0.1^{a}	0.12 ± 0.05^{a}	0.60 ± 0.03^{b}	1.18 ± 0.06^{c}	0.47 ± 0.05^{b}	1.7 ± 0.1^{d}	0.60 ± 0.05^{b}	0.12 ± 0.03^{a}
18:0	0.02 ± 0.02^{a}	0.03 ± 0.02^{a}	0.20 ± 0.03^{b}	0.62 ± 0.04^{c}	0.21 ± 0.02^{b}	0.71 ± 0.05^{c}	0.22 ± 0.02^{b}	0.06 ± 0.03^{a}
20:0	ND	0.01 ± 0.01^{a}	0.03 ± 0.01^{ab}	0.05 ± 0.02^{b}	0.02 ± 0.01^{a}	0.05 ± 0.02^{b}	0.03 ± 0.01^{ab}	0.02 ± 0.01^{ab}
22:0	ND	ND	0.01 ± 0.01	0.03 ± 0.02	ND	0.05 ± 0.03	ND	ND
Total	0.1 ± 0.1^{a}	0.15 ± 0.05^{a}	0.85 ± 0.06^{b}	1.9 ± 0.1^{c}	0.70 ± 0.03^{b}	2.5 ± 0.1^{d}	0.85 ± 0.05^{b}	0.20 ± 0.03^{a}

#1, batch 1; #2, batch 2; #3, batch 3; ND, not detected.
*The unit for contaminants is μg/cm^2 on paper or filter, values represent mean ± SD ($n = 3$), different superscripts indicate significant difference between groups, $P < 0.05$.

Table 3. Amount of contaminants released from collection papers during methylation process before and after soaking in 1% (v/v) H_2SO_4 in anhydrous methanol

		After soaking in Chloroform : Methanol (2:1) at room temperature			After soaking in 1% (v/v) H_2SO_4 in anhydrous methanol at 70°C		
	Before soaking	1 h	2 h	3 h	1 h	2 h	3 h
Sources of papers*							
Whatman glass fibre filter	0.1 ± 0.1	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.06 ± 0.03	0.03 ± 0.01	0.02 ± 0.01
Whatman Ion exchange paper	0.15 ± 0.05	0.08 ± 0.02	0.06 ± 0.02	0.05 ± 0.02	0.08 ± 0.03	0.06 ± 0.02	0.05 ± 0.03
Whatman 3 MM	0.85 ± 0.06^{a}	0.35 ± 0.02^{c}	0.18 ± 0.02^{d}	0.16 ± 0.02^{d}	0.51 ± 0.03^{b}	0.42 ± 0.02^{bc}	0.35 ± 0.03^{c}
Fluka kit	1.9 ± 0.1^{a}	0.82 ± 0.03^{c}	0.51 ± 0.05^{d}	0.42 ± 0.03^{d}	1.2 ± 0.1^{b}	0.80 ± 0.03^{c}	0.51 ± 0.05^{d}
Hemaspot-80	0.70 ± 0.03^{a}	0.27 ± 0.03^{c}	0.20 ± 0.02^{d}	0.15 ± 0.02^{d}	0.40 ± 0.05^{b}	0.31 ± 0.02^{bc}	0.27 ± 0.03^{c}
Whatman 903 paper #1	2.5 ± 0.1^{a}	1.1 ± 0.1^{c}	1.2 ± 0.1^{c}	1.0 ± 0.1^{c}	1.6 ± 0.1^{b}	0.80 ± 0.02^{d}	0.45 ± 0.02^{e}
Whatman 903 paper #2	0.85 ± 0.05^{a}	0.35 ± 0.02^{c}	0.25 ± 0.02^{d}	0.22 ± 0.01^{d}	0.55 ± 0.03^{b}	0.40 ± 0.03^{c}	0.31 ± 0.03^{c}
Whatman 903 paper #3	0.20 ± 0.03^{a}	0.11 ± 0.01^{bc}	0.08 ± 0.01^{cd}	0.09 ± 0.01^{cd}	0.14 ± 0.01^{ab}	0.09 ± 0.01^{cd}	0.07 ± 0.01^{d}

#1, batch 1; #2, batch 2; #3, batch 3.
*The unit for contaminants is μg/cm^2 on paper, value represent mean ± SD ($n = 3$), different superscripts indicate significant difference between groups, $P < 0.05$.

Table 4. Amount of potential contaminants from different sources

Contaminants corresponding to fatty acid peaks*	Nitrile glove	Latex glove	PE ziplock bag	Aluminum foil ziplock bag	Cellophane bag
16:0	0.005 ± 0.002	0.6 ± 0.02	0.02 ± 0.01	ND	ND
18:0	ND	0.6 ± 0.01	0.01 ± 0.01	ND	0.010 ± 0.002
18:1 n-9	ND	ND	0.31 ± 0.02	ND	ND
22:1 n-9	ND	ND	0.48 ± 0.03	0.32 ± 0.02	ND
Total	0.005 ± 0.002^{a}	1.20 ± 0.02^{b}	0.8 ± 0.05^{c}	0.32 ± 0.02^{d}	0.010 ± 0.002^{a}

ND, not detected.
*The unit for contaminants is μg/cm^2 on glass microfiber filter after wiped on the surface of gloves or bags, values represent mean ± SD ($n = 3$), different superscripts indicate significant difference between groups, $P < 0.05$.

2.3 Gas chromatography analysis

FAME were separated and quantified by using a GC (Hewlett–Packard 6890; Palo Alto, CA, USA) equipped with a BPX70 capillary column 50 m × 0.32 mm, film thickness 0.25 μm (SGC Pty Ltd., Victoria, Australia), PTV Injector and a flame ionisation detector (FID). The injector temperature was set at 250°C and the FID temperature at 300°C, a programmed temperature ramp (140–240°C) was used. Helium gas was utilized as a carrier at a flow rate of 35 cm/s in the column and the inlet split ratio was set at 20:1. The identification and quantification of FAME was achieved by comparing the retention times and peak area values of unknown samples to those of commercial lipid standards (Nu–Chek Prep Inc., Elysian, MN, USA) using the Hewlett–Packard Chemstation data system.

2.4 Statistical analyses

All statistical analyses were conducted using PASW Statistic 18. Values are expressed as mean ± standard deviation (SD). One-way ANOVA was used to determine the significant difference between percentages of fatty acids in total blood lipid, and $P < 0.05$ was used to determine statistical significance.

3 Results and discussion

3.1 Different results between blood spot samples and fresh blood

Analysis of 25 μL blood spotted on both ion exchange paper and glass microfiber filter produced identical results for fatty acid composition compared with the fresh blood measurement. The 25 μL blood spots on Whatman 3 MM paper showed similar fatty acid composition for the majority of the fatty acid classes, with the only exception of a higher percentage of palmitic acid (16:0). The 25 μL blood spots on the Fluka test card or Whatman 903 collection card contained a significantly lower percentage of omega-3 fatty acids and arachidonic acid (AA, 20:4 n-6) in total blood lipids when compared with the fresh blood measurement (Table 1). This apparent reduction in the proportion of omega-3 and AA in blood spot samples was offset by a significant increase in the percentages of the saturated fatty acids, palmitic acid, and stearic acid (18:0) measured in these samples. When the volume of blood spot was increased to 50 μL, the fatty acid composition measured from the blood spots collected on either Whatman 3 MM paper or Fluka test card was not significantly different from those in fresh blood, however, analysis of 50 μL blood spots on the Whatman 903 collection card still produced significantly different fatty acid profile to that in fresh blood (Table 1).

The blood spot analysis technique is regarded as a quick and inexpensive method for determining the total lipid fatty acid composition in whole blood, and its effectiveness has been validated in a small number of clinic trials [11, 12]. However, none of previous validation studies have assessed the accuracy of this method in individuals in comparison to direct transmethylation of fresh blood for determination of fatty acid composition in whole blood. The results presented here clearly indicate that the fatty acid status test results from blood spot samples can vary substantially from those obtained from fresh whole blood. The Fluka blood collection kit and Whatman 903 specimen collection card are commercial products which are widely used for blood collection for a range of analyses, including fatty acid analyses, and Whatman 3 MM chromatography paper is also frequently used as a blood collection paper in clinical trials [7, 8]. However, low volumes of blood (25 μL) spotted on any of these three types of paper produced dubious fatty acid results in comparison to those obtained from the direct assessment of LCPUFA from a drop of whole blood. Our data clearly demonstrated that using certain types of collection papers may result in the inaccurate determination of the amount of the respective classes of fatty acids as a proportion of total lipids, especially when the volume of blood collected on paper is low. This appears to be explained in part by the presence of contaminants released from collection papers during the methylation procedure (Table 2). Thus, caution needs to be exercised when selecting the type of paper used in the collection of blood spot samples for fatty acid analyses.

3.2 Contaminants from different papers

We found that the two major contaminants in all types of collection paper showed the same GC retention times as the 16:0 and 18:0 saturated fatty acids (Table 2). This explains the increase in the percentages of 16:0 and 18:0 saturated fatty acids that was detected in blood spot samples when compared with those in fresh blood. Furthermore, the total amount of contaminants varied between different types of collection paper. For example, the Whatman ion exchange paper had only 0.12 μg/cm^2 of contaminants, whereas the Whatman 903 paper batch 1 had 20 × this amount. It is also noteworthy that different batches of the same type of paper have the potential to release different amount of contaminants. For example, the contaminants released from the three different batches of Whatman 903 paper analyzed in this study ranged from 0.20 μg/cm^2 to 2.5 μg/cm^2.

Previous studies conducted on Fluka paper and Whatman 903 paper claimed that no contaminant peak was observed in GC after processing the collection paper alone [5, 13]. However, we found contaminant peaks in all types of collection paper tested, especially for Whatman 903 sample collection paper batch 1 which was found to release ~5.6 μg of contaminants from a 1.5 × 1.5 cm square of the collection paper. This level of contaminants, particularly given their

concordance with the retention times of that fatty acids 16:0 and 18:0, resulted in deviations of ~7% in the relative proportion of different fatty acid classes in comparison with those in whole blood in a 25 μL blood spot, which clearly has the potential to have major impacts on the results of fatty acid measurements and their clinical interpretation. It may be the case that small quantities of contaminants in the collection paper do not significantly alter the fatty acid composition test result. For example, Whatman ion exchange paper and Whatman glass microfiber filter showed identical results in fatty acid composition with that of fresh blood (Table 1). However, for those papers which contain high amounts of contaminants, such as the Fluka test kit and Whatman 903 sample collection paper batch 1, the fatty acid results were obviously different to fresh blood when the volume of blood collected was small as described above (Table 1).

Contaminating substances released from Japanese blood collection papers (Toyoroshi, Japan) was first reported by Nishio et al. [14], and the structures of contaminants released from their collection paper were elucidated as 16:0 and 18:0 saturated fatty acids . Another study using Whatman 3 MM paper as a blood collection paper to test fatty acid composition of glycerolipids in blood and breast milk suggested a method to wash out the contaminants from collection paper by soaking the paper for a few minutes in acetone prior to use [15]. However, the structure of contaminants in Whatman 3 MM paper was not discussed. The structure of the contaminants in the collection papers we tested was unclear. They may be resin acids or fatty acids, because both are present in wood, from which these papers are ultimately synthesized, either as free acids or various esters [16]. Resins and fatty acids from processed water samples obtained from a paper mill, including 16:0 and 18:0 saturated fatty acids have been reported by others [17]. Thus, it appears that these acids or their esters can remain as impurities in the final paper product and lead to the introduction of contaminants when assessing fatty acid composition of blood spots collected on these papers.

3.3 Effect of pre-soaking collection paper in methylating agent

About 60% of the contaminants that were present before soaking were retained in collection papers after 2 h of soaking in methylating agent (1% H_2SO_4 in methanol) at 70°C (Table 3). Further extend the incubating time to 3 h washed out nearly 80% of the contaminants from all types of papers. However, it also destroyed the texture of collection papers and made these unsuitable for blood collection.

Soaking the collection papers in chloroform: methanol (2:1) for 3 h washed out nearly 80% of the contaminants for most of the blood collection paper except Whatman 903 sample collection paper batch 1 which showed a residue of around 40% of contaminants (Table 3). Thus, while pre-soaking may remove some of the contaminants from collection papers, it is not an appropriate or practical way to eliminate contamination.

3.4 Potential contaminants from other sources

Other sources of contaminants were tested in our laboratory by touching papers to a consistent surface area of ~10cm^2 of materials used for the processing and storage of blood spots, including latex gloves, nitrile gloves, PE ziplock bags, aluminum foil ziplock bags, and cellophane bags (Table 4). Latex gloves contained a significant amount of contaminants however there was virtually no contamination detected from the nitrile glove. The PE bag and the aluminium bag each contained large amounts of contaminants which showed the same GC retention times as oleic acid (18:1 n-9) and erucic acid (22:1 n-9), however, touching the papers with the cellophane bag did not result in any detectable contamination.

Latex gloves, PE bags, and aluminium bags are widely used in many laboratories. There have been no previous studies focussing on latex gloves as a source of contamination in any experiment, with existing investigations reporting only that latex gloves can release endotoxins which may be associated in skin irritation and/or allergy [18]. However, our experiments have indicated that even these widely used laboratory items have the potential to release hydrophobic contaminants and could contribute to alteration in the results of blood fatty acid composition tests, especially when the blood sample volume is low. Thus, to minimize potential contaminant problems from gloves and bags, we recommend that it is important to use nitrile gloves and cellophane bags during collection, processing, and storage of the blood spot samples.

4 Conclusions

All types of collection paper release contaminants and these contaminants may alter the results of analyses of blood spot samples, particularly when the volume of the blood collected on paper is low. Due to the variation in contaminant levels and in the efficiency of pre-soaking in reducing these levels between different type/batches of collection papers, either pre-soaking the paper or subtracting a blank value are not fully effective or practical strategies for removing the contaminants from paper. Therefore, the choice of a collection paper which contains minimum contaminants is important for the rapid fatty acid composition test. We were unable to detect any significant contaminants from the Whatman ion exchange paper and Whatman glass microfiber filter, and they showed identical fatty acid composition results with that in fresh blood even when the amount of blood on paper was low (25 μl). Furthermore, our experimental result also revealed that using nitrile gloves and cellophane bags can reduce potential contamination during blood spot sample collection, processing, and storage.

Since Whatman ion exchange paper releases much lower levels of contaminants than the Fluka collection card and Whatman 903 paper, it may have the potential to replace Fluka collection card and Whatman 903 paper in the blood fatty acid composition test to provide more accurate results. Moreover, another recent study by our group [9] has validated the use of Whatman ion exchange paper as a reliable blood collection paper for the applications in clinical trials which are aimed at measuring fatty acid profiles in dried blood spot samples.

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