In vitro detection of bacterial contamination in platelet concentrates by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry: a preliminary study

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Abstract

Purpose. Platelet concentrates are at risk of transfusion-related sepsis. The microbial detection methods currently available have reached their limits, as they do not completely prevent transfusion-related bacterial contamination. The aim of this study was to develop a new strategy to detect the risk of platelet transfusion-related bacterial contamination using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

Methodology. In vitro, platelet concentrates were seeded with known concentrations of bacterial strains. Protein mass profiles were acquired by using a Microflex MALDI-TOF instrument. Dedicated ‘Platelet’ software was used as a spectrum subtraction tool to reveal specific peaks caused by the presence of pathogens in samples.

Results. The MALDI-TOF spectra of platelets were characterized and the reproducibility over time, regardless of the blood donor, was demonstrated with a positive predictive value of 100 %. In addition, the negative predictive value of the total number of specific peaks to predict contamination was 100 %.

Conclusion. Detecting bacteria in platelet concentrates using the MALDI-TOF approach and analysing spectra with the Platelet software present the advantage of combining the precocity of results and sufficient sensitivity (10 c.f.u. ml⁻¹). Further research will be conducted to compare this novel method with the current conventional method in order to validate our results, the objective being to reduce the risk of platelet transfusion-related bacterial contamination.

INTRODUCTION

Bacterial contamination of platelet concentrates (PCs) is an ongoing problem causing significant transfusion-associated morbidity and mortality with serious, and even fatal, septic reactions in patients despite all interventions in most countries [1, 2]. It is generally accepted that bacterial contamination occurs in about 1/2000 transfused PCs [3–5]. Indeed, the room temperature conditions required for preserving functional platelets during storage favour the proliferation of bacteria. However, the clinical expression of infection depends on the virulence of bacterial strains, the amount of bacteria transfused and the patient’s immunological status. Strategies to reduce platelet transfusion-related bacterial contamination remain a priority, and potential interventions include improvements in donor selection, optimal skin disinfection and diversion of the first aliquot of whole blood [6, 7].

Bacterial detection is commonly used in most countries. However, current bacterial screening methods for PCs display different levels of sensitivity and none is likely to detect all pathogens [8]. The widely used BacT/Alert is a colorimetric culture method based on the detection of carbon dioxide produced by micro-organisms. The risk of septic reactions is not completely prevented by the implementation of this detection method and there is...
convincing evidence that false negatives occur [1, 8–11]. Indeed, PCs could be administered before the BacT/Alert result is known. Indeed, the maximum storage time of PCs is 5 days.

Among alternative strategies, the Haemonetics e-BDS (Pall Corporation) detects bacterial growth by the drop in oxygen levels in the medium. But only aerobic bacteria can be detected, which generates a risk of false-negative results [12]. More recently, a rapid test to detect bacteria has been developed. The Pan Genera Detection test is a rapid qualitative immunoassay that can detect aerobic as well as anaerobic Gram-positive and Gram-negative bacteria [13]. Different studies have shown its lack of sensitivity and specificity, and have suggested that it should only be used in addition to other techniques such as automated growth [14]. Other rapid detection methods exist, such as a flow cytometry, which is now applied in German centres [15].

In this context, the need to develop another effective strategy for detecting bacterial contamination in PCs is still relevant.

Many microbiology laboratories use matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) [16, 17] as it provides reliable bacterial identification from colonies in a record time and at low cost [17–19]. In addition, MALDI-TOF MS enables direct identification of micro-organisms from blood cultures [20–22] and urine specimens [23, 24].

Based on these findings, the aim of this study was to evaluate the suitability of MALDI-TOF to precociously detect in vitro bacterial contamination of PCs.

METHODS

PCs

Whole blood (WB) was collected from healthy donors by the investigators working at the French National Blood collection centre (EFS, Marseille, France). All participants gave informed written consent. The blood donor selection criteria are described in the Public Health Code (Code de la santé publique, decree of January 12, 2009). The protocol was approved by the IHU Méditerranée Infection ethics committee (reference 2016-002). All samples were completely de-identified, with all demographic data and health information removed.

Five hundred donated WB units (467±7 ml) were collected in accordance with standard operating protocols. On day 0, the sample was collected in a quintuple bag (DGR7542; Fenwal) and stored overnight at 21±3 °C. On day 1, the WB was centrifuged at 64 000 r.p.m. (=6010 g) for 15 min (acceleration 9, break 5) using a Heraeus Cryofuge 6000 centrifuge. The centrifuged products were then separated on automatic presses (Compomat G4, NBPI Fresenius, and top/bottom separation). Five ABO isogroup buffy coats and 280 ml Intersol (Fenwal) platelet additive solutions (PASs) were pooled using the integrated pooling system in the kit. The pools were then placed in an automatic device which processes sixuffy coat pools at the same time. Each cycle carries out the simultaneous centrifugation, separation and leukoreduction of products within 12 min. One hundred PC units were obtained. The mean platelet content in routine conditions for TACSI system produced leukoreduceduffy coat platelets concentrates (LR-BCPC) is 4.5±0.4 10^{11} with a mean volume of 333±11 ml.

Bacterial strains and culture conditions

The study was conducted with the seven micro-organisms frequently associated with the contamination of PCs [25]. The strains, isolated from patient specimens at our hospital, were characterized in our microbiological laboratory and deposited in the CSUR collection (Collection de Souches de l’Unité des Rickettsies).

Following the recommendations of the ISBT on transfusion-transmitted infectious diseases [25], we cultured Staphylococcus epidermidis (CSUR P2203), Streptococcus pyogenes (CSUR P576), Klebsiella pneumoniae (CSUR P2205) and Escherichia coli (CSUR P2202). Three other strains, Staphylococcus aureus (CSUR P2188), Providencia stuartii (CSUR P2204) and Pseudomonas aeruginosa (CSUR P2201), were also cultured.

Bacterial suspensions were obtained from cultures placed overnight on 5 % sheep blood agar plates (bioMérieux). The number of c.f.u.s was divided by the product of the dilution factor. Serial dilutions of these suspensions yielded concentrations from which small aliquots were inoculated. The actual inoculum was confirmed by quantitative culture of the serial dilutions on sheep blood agar.

Samples of 900 µl PC were seeded with 100 µl of the different suspensions of each strain at increasing concentrations of 10^{1} to 10^{7} c.f.u. ml^{-1}. The mixture was then incubated with and without tripticase soy broth (TSB) medium. After 16 h, the mixtures were subjected to the MALDI-TOF procedure. In parallel, a quality check for purity was performed by plating 100 µl of PCs on 5 % sheep blood agar plates and colonies were identified using MALDI-TOF MS [17]. One millilitre of unseeded PC was used as a negative control. To ensure the sterility of PCs before inoculation, they were cultured onto solid agar media according to standard microbiological procedures.

MALDI-TOF procedure

Sample preparation

Samples of 900 µl PC from healthy whole units were placed in a 2 ml sterile Eppendorf tube with a pipette tip. Then, 100 µl of bacterial solution of a known concentration (10^{1}, 10^{2}, 10^{3}, 10^{4} and 10^{5} c.f.u. ml^{-1}) was added and incubated in a shaker incubator at ~150 r.p.m. at 37 °C for 16 h with and without 1000 µl of TSB. One millilitre of PC was used as a negative control. Samples were centrifuged for 5 min at 13 000 g. The supernatant was then removed and the pellet was
suspended in 700 µl of water and 300 µl of saponin (Sigma Aldrich), and centrifuged for 5 min at 13 000 g. The same procedure was then performed with 1 ml of sterile water. The supernatant was discarded and the pellet was air-dried for 10 min at room temperature. Subsequently, 30 µl of formic acid (70 % v/v) was added to the pellet and mixed thoroughly before the addition of 30 µl acetonitrile to the mixture, which was centrifuged for 5 min at 13 000 g. One microlitre of the supernatant was deposited on a spot of the steel target and air-dried at room temperature. The extraction time for 48 samples was 2 h. Each sample was overlaid in duplicate with 1 µl of the matrix solution (saturated solution of alpha-cyano-4-hydroxycinnamic acid; Sigma Aldrich) in organic solvent (50 % acetonitrile and 2.5 % trifluoroacetic acid) and air-dried (Fig. 1).

MALDI-TOF parameters

Protein mass profiles were acquired using a Microflex MALDI-TOF mass spectrometer (Bruker Daltonics) with FlexControl software (Bruker Daltonics). We performed measurements in positive linear mode at a laser frequency of 200 Hz within a mass range of 2–20 kDa. The IS1 voltage was 20 kV, the IS2 voltage was maintained at 18.05 kV, the lens voltage was 6 kV, and the extraction delay time was 120 ns. For each spectrum, 2000 laser shots were performed and analysed (20 × 100 laser shots). The spectra were calibrated externally using the standard calibration mixture (E. coli extracts including the additional proteins RNase A and myoglobin; Bruker Daltonics). For each bacterial strain, the whole cell's protein profile was determined in quadruplicate. The raw spectra obtained for each isolate were processed using FlexAnalysis version 3.3 and MALDI Biotyper version 3.0 software (Bruker Daltonics) and analysed by standard pattern matching (with default parameter settings) against the MALDI Biotyper database. The run time of the MALDI-TOF with 96 spots was 2 h (48 samples in duplicate).

Spectral analysis and MALDI-TOF MS identification

To study the reproducibility of the PC spectra, the average spectral profiles obtained from the four spots for each specimen (100 PC units with determinate ABO blood groups) were analysed and compared using FlexAnalysis software (Bruker Daltonics). Identification scores were assigned using the following scoring parameters: a score ≥1.9 indicated species identification; a score of 1.7–1.9 indicated genus identification; and a score <1.7 indicated no identification. A sample was considered to be correctly identified by MALDI-TOF when two spectra had a score ≥1.9 [17].

Platelet software

As the assays were performed in a complex medium including platelets and bacteria, the association of platelet and bacterial spectra impeded bacterial identification by spectral homology. These observations led us to develop software to subtract spectrum areas and to highlight the differential peaks between two spectra. The software subtracts the common peaks, revealing specific peaks related to microbial contamination. Therefore, we also considered the differential number of peaks, which were regarded as specific peaks. The first step consists of the detection of significant peaks in all spectra using a normalization of the spectra as well as the suppression of background noise.

A peak is defined and retained when peak intensity is five times higher than the upper extreme whisker value estimated from a boxplot of all peaks in the spectra: (upper_whisker=(upper_quartile)+1.5×IQR with IQR= (upper_quartile)–(lower_quartile). In the second step, peaks are matched between both spectra by comparing the reciprocal presence or absence of peaks in an x-axis window slide of 0–20 kDa. The total and unique number of peaks is subsequently reported for each spectrum. The time of analysis of 48 samples is 20 min and the results do not require further manipulation. The results are retrieved in an Excel table. The software program is freely available and the source code is provided in the supplemental data file (File S1, available in the online Supplementary Material). The software runs under Windows and Linux operating systems and requires previous installation of Python 2.7, Matplotlib and NumPy.

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**Fig. 1.** Flow chart describing the detection of bacteria from contaminated platelets concentrates samples.
Statistical analyses were performed using GraphPad Prism software, version 6. Quantitative variables are reported as mean ± standard deviation.

An ANOVA test was used for intergroup comparisons of the general data. A P-value of less than 0.05 was considered to be statistically significant.

The area under a receiver operating characteristic (ROC) curve was used to determine the optimal cut-off value for detecting bacterial contamination with the best sensitivity and specificity. A P-value lower than 0.05 was considered to be statistically significant.

### RESULTS

#### Detection of bacterial contamination in PCs by MALDI-TOF

Assays were performed on MALDI-TOF after 16 h of incubation with and without TSB medium and spectra profiles were analysed using FlexAnalysis software (n=10 for each strain in quadruplicate for each concentration).

When seeded PCs were incubated without TSB medium, spectra profiles were comparable to the negative control regardless of concentration, seeded strain or blood group. (Fig. 2a). As shown in Fig. 2(b), when PCs were incubated with TSB, specific peaks were observed after 16 h of incubation at 37°C regardless of concentration, seeded strain or blood group.

### Table 1. Identification rate using Biotype for seven reference bacterial strains at 10, 10^2 and 10^7 c.f.u. ml⁻¹ in PCs (n=10 for each strain in quadruplicate for each concentration)

A score >1.9 indicated species identification, a score of 1.7–1.9 indicated genus identification and a score <1.7 indicated no identification [17]. The percentage represents the detection rate of matched pattern.
Analyses of all spectra were also obtained by using FlexAnalysis software and analysed by standard pattern matching against the MALDI Biotyper database. Table 1 summarizes the percentage of identification scores obtained with the Biotyper software score. For $10^7$ c.f.u. ml$^{-1}$, spectrum analysis enabled bacterial identification regardless of the strain. For $10^2$ c.f.u. ml$^{-1}$, Biotyper analysis was able to detect *E. coli*, *K. pneumoniae*, *Staph. aureus*, and *Ps. aeruginosa*. However, *Strep. pyogenes*, *Staph. epidermidis* and *Pr. stuartii* were not identified. Interestingly, when the assays were performed at 10 c.f.u. ml$^{-1}$ before inoculation, only *E. coli* and *Staph. aureus* were detected. Under these analysis conditions,
bacterial contamination was not identified by standard pattern matching against the MALDI Biotyper database.

**Platelet software led to bacterial detection**

In order to carry out the comparative analysis of spectra from different samples, we have developed a new method based on peak identification and matching.

If identification failed to detect bacterial contaminants at the species level, the software was launched, using the spectra obtained by the MALDI, and specific peaks were detected by comparing the spectra generated between two classes. Any significant changes indicated a possible bacterial contamination of PCs. Fig. 3(a) is representative of classes. Any significant changes indicated a possible bacterial contamination of PCs based on MALDI-TOF fingerprinting. The optimal threshold of this test is shown in Fig. 3(c).

The bacterial contamination of PCs remains a major infectious risk in developed countries. Despite the implementation of preventive measures, this risk persists but at a much lower level than before, with a mortality risk of <1 per million [2]. Different screening methods have been proposed to detect the risk of transfusion-associated septic reactions. However, these systems fail to meet all the requirements of sensitivity, specificity, and rapidity [26].

**DISCUSSION**

We have developed a new strategy for detecting in vitro bacterial contamination of PCs based on a MALDI-TOF approach combined with home-made Platelet software. Our results are reliable as they have been performed in quadruplicate and verified by using the culture method. The major advantage of this in vitro method compared to currently available techniques is that it combines faster results (16 h) and sufficient sensitivity (100 % sensitivity at 10 c.f.u. ml⁻¹ before the inoculation).

Table 2. Mean±SD of number of specific peaks obtained with the Platelet software after contamination of PCs and incubation with TSB for 16 h with seven strains at five different concentrations

For each concentration and for each strain, 30 assays were performed in quadruplicate.

Table 3. Positive and negative predictive value, sensitivity and specificity of number of specific peaks obtained by using the Platelet software

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**Table 2.** Mean±SD of number of specific peaks obtained with the Platelet software after contamination of PCs and incubation with TSB for 16 h with seven strains at five different concentrations

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Number of specific peaks (n=30, in quadruplicate)</th>
<th>10⁻⁷ c.f.u. ml⁻¹</th>
<th>10⁻⁸ c.f.u. ml⁻¹</th>
<th>10⁻⁹ c.f.u. ml⁻¹</th>
<th>10⁻¹⁰ c.f.u. ml⁻¹</th>
<th>10⁻¹¹ c.f.u. ml⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>K. pneumonia</em></td>
<td>18.5±4.5</td>
<td>31.43±7.0</td>
<td>33.33±9.5</td>
<td>37±7.2</td>
<td>41.1±6.1</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>22.1±4.0</td>
<td>33±5.9</td>
<td>34.6±7.0</td>
<td>39±8.3</td>
<td>43±7.7</td>
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</tr>
<tr>
<td><em>Strep. pyogenes</em></td>
<td>15.7±3.4</td>
<td>24.5±4.2</td>
<td>28±5.0</td>
<td>30.5±4.5</td>
<td>36.6±4.7</td>
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</tr>
<tr>
<td><em>Staph. epidermidis</em></td>
<td>16.5±3.0</td>
<td>26±3.8</td>
<td>31.3±5.8</td>
<td>32.5±7.1</td>
<td>37.6±3.6</td>
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<tr>
<td><em>Staph. aureus</em></td>
<td>19.5±2.1</td>
<td>22.1±5.0</td>
<td>35±7.2</td>
<td>40±5.6</td>
<td>43.5±7.2</td>
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<tr>
<td><em>Pr. stuartii</em></td>
<td>15.2±3.1</td>
<td>19.2±4.3</td>
<td>31.5±5.5</td>
<td>34±5.5</td>
<td>36±5.5</td>
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<tr>
<td><em>Ps. aeruginosa</em></td>
<td>18.1±4.0</td>
<td>26.1±3.5</td>
<td>32±5.4</td>
<td>42±4.8</td>
<td>44.9±6.9</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.16±3.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>0.001 c.f.u. ml⁻¹</th>
<th>0.01 c.f.u. ml⁻¹</th>
<th>0.1 c.f.u. ml⁻¹</th>
<th>1 c.f.u. ml⁻¹</th>
</tr>
</thead>
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<td>18.5±4.5</td>
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<tr>
<td>Control</td>
<td>4.16±3.2</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Table 3. Positive and negative predictive value, sensitivity and specificity of number of specific peaks obtained by using the Platelet software

**Table 3.** Positive and negative predictive value, sensitivity and specificity of number of specific peaks obtained by using the Platelet software

<table>
<thead>
<tr>
<th>Test</th>
<th>Infected</th>
<th>Non-infected</th>
<th>Predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive test</td>
<td>4200 (True positive value)</td>
<td>0 (False positive value)</td>
<td>Positive predictive value 4200/4200=1</td>
</tr>
<tr>
<td>Negative test</td>
<td>0 (False negative value)</td>
<td>640 (True negative value)</td>
<td>Negative predictive value 640/640=1</td>
</tr>
</tbody>
</table>

Sensitivity: 4200/4200=100%
Specificity: 640/640=100%
Positive Predictive Value: 4200/4200=100%
Negative Predictive Value: 640/640=100%

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For each concentration and for each strain, 30 assays were performed in quadruplicate.
MALDI-TOF has recently emerged as a powerful tool for rapid bacterial identification based on peptide spectra and has been successfully used for the routine identification of grown and subcultured colonies given its low cost of consumables. A protocol for bacterial identification directly from blood culture [27] and mono-microbial urine samples was subsequently developed [28]. Consequently, bacterial identification in PCs would appear possible, even if it requires developing sample preparation processes. Herein, our protocol includes 16 h of incubation with TSB medium followed by 2 h of sample preparation. For safety reasons, when a unit of PC is detected positive, the microbiology laboratory informs both the institution and the unit which administered the PC. We tried to reduce the incubation time to 8 h, which lowered the sensitivity of the method (data not shown). As a consequence, this did not allow for the detection of some species with an acceptable sensitivity, notably Staph. epidermidis and for lower concentrations of any strain. However, considering that detection by other processes is conventionally carried out after 24 h of agitation at 22 °C, this method met our expectations in terms of delay.

The other challenge of our strategy was to detect low bacterial loads in a complex environment. Indeed, if it is generally accepted that serious reactions at the time of transfusion are observed for bacterial concentrations greater than or equal to 10^3 c.f.u. ml^-1 [5], blood products could be contaminated by skin flora with very small amounts of bacteria (estimated between 10 and 10^2 bacteria) [29].

Here, the FlexAnalysis program failed to perform genus identification and could also have given false negative results for concentrations below 10^2 c.f.u. ml^-1.

To manage this problem, we developed an original spectrum analysis system: ‘Platelet’ software. This subtracts the respective total number of peaks of a known analysed spectrum from a reference platelet spectrum, as previously established in this work. This is an original concept, which takes into account all bacterial and platelet peaks, i.e. all of the resulting spectra. The appearance of significant variation between the two spectra (reference spectrum/analysed PC spectrum) shows bacterial contamination. Indeed, the use of a negativity threshold >1 is associated with PC contamination with a negative predictive value of 100 %. Furthermore, the Platelet software does not require a database, unlike Biotyper.

One of the main limitations of this study is that the Platelet software is not able to provide identification at the species level. However, the main objective of this work was above all to detect contamination in PCs and remove the unit from the circuit to identify the species.

To the best of our knowledge, this is the first time that MALDI-TOF has been used to detect bacterial contamination in PCs. These results met our objectives: in addition to the sensitivity criteria, we were able to address the issue of early detection. The method described here is fast, very robust and able to cope with large numbers of samples. As a next step, it would be interesting to try this approach on a full PC unit under real conditions. Despite promising results, this method has to be compared with conventional techniques in real-world settings. In practice, this early detection leads to the withdrawal of PCs and also prevents the transfusion of contaminated PCs.

**Conclusion**

By using MALDI-TOF MS, we were able to detect 100 % of PC contamination in less than 20 h. This method could potentially be used routinely to exclude contaminated PCs, thus improving blood safety.

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**Acknowledgements**

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**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**Ethical statement**

All procedures in this study involving human participants were conducted in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethics standards. The protocol was approved by the IHU Méditerranée Infection ethics committee (Reference 2016-002). Informed consent was obtained from all individual participants included in the study.

**References**


