Effect of adhesive properties of buffy coat on the quality of blood components produced with Top & Top and Top & Bottom bags

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Introduction
Good blood banking practice requires that every effort should be made to detect any deviation in blood components and to identify the opportunities for quality improvement. The "Italian Standards in Transfusion Medicine", edited by the Italian Society of Transfusion Medicine and Immunohaematology (Società Italiana di Medicina Trasfusionale e Immunohaematologia, SIMTI)1, stipulates that all performance defects must be recorded and reported to the Quality Assurance Manager. If the defect or the low performance appears to be batch-related, all the bags and the blood collected in them must be set aside for further investigation.

Our Transfusion Medicine Service produces more or less 20,000 red cell concentrates without buffy coat (RCC) per year, using quadruple Top & Top (T&T) bags with a small-size pocket for the buffy coat (BC). Alternatively to T&T, the specialist literature indicates Top & Bottom (T&B) bags as the most suitable for obtaining RCC of excellent quality2,3. Since we already use quadruple T&B bags with an incorporated soft leucoreduction filter (Fresenius T&B CQ32250; Fresenius HemoCare Italia, Modena, Italy) for the production of pre-storage leucoreduced concentrated erythrocytes, needed primarily for multiply transfused patients, we wanted to try similar bags, but without a pre-installed filter, in order to verify whether it would be possible to completely replace the traditional T&T bags in our current organisation. The final objective was an improvement action that should simplify operational modalities and, primarily, improve the quality of blood components.

Materials and methods
Twenty-one whole blood (WB) donations, corresponding to the average number tested monthly for quality control, were collected in our Transfusion Medicine Service using new quadruple T&B PQ32150 bags, provided to us for test purposes by Fresenius HemoCare Italia. The bags were assessed and compared to the routine method with regards to product quality and operational requirements.

Background. The Transfusion Medicine Unit of Reggio Emilia currently collects whole blood using conventional quadruple Fresenius Top & Top bags. In this study, new Fresenius Top & Bottom bags were assessed and compared to the routine method with regards to product quality and operational requirements.

Materials and methods. Twenty-one whole blood units were collected with both the new and the traditional bags, and then separated. Quality control data were evaluated and compared in order to estimate yield and quality of final blood components obtained with the two systems. We collected other bags, not included in the ordinary quality control programme, for comparison of platelet concentrates produced by pools of buffy coat.

Results. Compared to the traditional system, the whole blood units processed with Top & Bottom bags yielded larger plasma volumes (+5.7%) and a similar amount of concentrated red blood cells, but with a much lower contamination of lymphocytes (−61.5%) and platelets (−86.6%). Consequently, the pooled platelets contained less plasma (−26.3%) and were significantly richer in platelets (+17.9%).

Discussion. This study investigated the effect of centrifugation on the adhesiveness of the buffy coat to the bag used for whole blood collection. We analysed the mechanism by which this undesirable phenomenon affects the quality of packed red blood cells in two types of bags. We also documented the incomparability of measurements on platelet concentrates performed with different principles of cell counting: this vexing problem has important implications for biomedical research and for the establishment of universal product standards. Our results support the conclusion that the Top & Bottom bags produce components of higher quality than our usual system, while having equal operational efficiency. Use of the new bags could result in an important quality improvement in blood components manufacturing.

Keywords: Top & Bottom bag, Top & Top bag, blood buffy coat, adhesiveness, accumulated centrifugal effect.
HemoCare Italia. In the same month, for purposes of comparison, 21 traditional T&T Fresenius T2466 quadruple bags were selected from among those from provincial collection centres for routine quality control, taking care to balance the proportion of males and females in the two groups.

The donations were processed within 6 hours of collection to produce RCC, type B fresh-frozen plasma (FFP) and BC suitable for production of platelet pools.

Within 24 hours of the WB collection, we also produced 21 platelet pools assembled exclusively with 5 BC from new T&B bags (from a total of 105 donors) and, for purposes of comparison, 21 other pools exclusively produced with 6 BC from traditional T&T bags (from a total of 126 donors).

All the materials employed were medical devices authorised for both trade and clinical use and not a single blood component was sacrificed for the sake of study. Each donor provided informed consent for donation and examination.

**Analyses and differences between the two types of bags**

For a better understanding of the different performances of the two types of WB bags, it is important to highlight their similarities and differences (Figure 1).

The plastic material used is identical: laminated polyvinylchloride with phthalates, specifically di(2-ethylhexyl)phthalate. The surface finishing of the plastic film is designed to reduce adhesion of platelets and simplify withdrawal from the centrifuge drum. Each drawing kit allows the collection of 450 mL of whole blood in 63 mL of citrate-phosphate-dextrose anticoagulant and the addition of 100 mL of sodium chloride, adenine, glucose and mannitol (SAG-M) as a preservative to the condensed erythrocytes.

In the T&B configuration, the RCC satellite container is linked to the lower part of the mother bag by means of a break-off septum, and already contains the SAG-M preservative solution, while the plasma container is linked by a similar septum to the upper part. The squeezing of erythrocytes and plasma occurs at the same time, and the BC remains in the centrifuged bag. All the bags in the kit are the same size and can hold over 500 mL of liquid. The fourth bag is used for the production of platelet concentrates from platelet-rich plasma or BC: in our case it was discarded.

Differently, in the case of WB bags with a T&T configuration, all the satellite containers are linked to the mother bag by means of a single valve located on the upper side. In two of these containers the plasma, and then the BC, are squeezed, while the erythrocyte concentrate remains in the centrifuged bag. The SAG-M contained in the third satellite bag is added at the end of the separation. The container receiving the BC is a mini-bag that can hold approximately 120 mL of liquid.

**Centrifugation of the mother bag**

The WB was centrifuged in two Sorvall RC12BP centrifuges (Thermo Scientific, Milan, Italy), which automatically calculate the accumulated centrifugal effect (ACE) of each work-cycle. ACE is a non-dimensional number which measures the area under the curve of the function rpm vs centrifugation time from start to stop, thus including the significant contribution of the acceleration and deceleration phases.

ACE represents the reference parameter when it is necessary to optimise a centrifugation protocol to obtain products with constant and defined quality features because it accurately measures the total centrifugal effect experienced by the suspended particles during centrifugation. A further advantage is provided by a simple transformation coefficient, which considers the radius of the rotor and the terrestrial gravity acceleration: it is possible to transform the ACE into total centrifugal force (TCF) which, by integrating the relative centrifugal force (RCF) over time, allows the effects of centrifugation using rotors and centrifuges of different types to be replicated.

Using the experience previously acquired by studying the influence of ACE in the production of platelet gels for topical use, we have established that the optimal ACE is $61.0 \times 10^6$ for traditional T&T bags and $77.0 \times 10^6$ for the T&B test bags to obtain the maximum yield in FFP volume, compatibly with an acceptable contamination of the RCC by white blood cells and platelets and without excessive compression or shear stress to the red blood cells.

**Separation of blood components and buffy coat assembly**

The WB was separated with four automatic NPBI CompoMat® G4 separators (Fresenius Kabi Italia, Verona, Italy). The separation process was completely automated and took place under the supervision of an operator, who intervened only in the initial and final phases.
The programmes used were customised for each type of bag and optimised to produce about 60 mL of BC with a constant haematocrit (about 30% for the T&T system and 35% for the T&B system), suitable for platelet-pool production. The final FFP and RCC volumes, on the other hand, depend on the donor’s haematocrit: inversely for the former and directly proportional for the latter.

To produce the platelet pools, 5 BC (from T&B bags) or 6 BC (from T&T bags) were pooled with the OrbiSac System (TerumoBCT, Rome, Italy) and mixed with 300 mL of MacoPharma SSP adjuvant solution (MacoPharma Italia, Milan, Italy) for preservation up to 5 days after collection with continuous stirring at 22 °C.

The average percentage of residual plasma in the final platelet pool was the same as that in the OrbiSac pooling bag before the centrifugation: around 46% of the total volume of liquid phase (plasma and SSP) for the control bags and around 39% for the test bag (MacoPharma recommends not dropping below 30%). The residual plasma volume can be estimated by multiplying the final volume of the platelet pool by that percentage.

Leucocytes are depleted directly on the OrbiSac with an in-line filter during processing.

**Quality control analysis and measurement limits**

In order to take the measurements we proceeded as for normal production checks. Significant samples of WB, RCC and platelet pool were taken from thermally welded segments, after thorough mixing of the bag followed by stripping of the sampling pipe at least five times. The content of each segment was immediately transferred into a blood counting vial containing K$_2$-EDTA, identified and carefully mixed before measurement by means of an automated haematology analyser (CELL-DYN Sapphire, Abbott Italia, Latina, Italy).

For the FFP only the recovery volume was compared, calculated by the net weight.

All measurements were verified and considered reliable. However, it is important to underline that the technical manuals of the blood cell counters usually report only the background limits of the complete blood count from patients, while the true limits of detection and quantification of different types of biological matrices are unknown in most cases. As we did not know the precise limits of the equipment for the analysis of the concentrated blood components, in our specific case and based on our experience, we used caution in evaluating the low values detected for platelets and residual white blood cells contained in RCC.

Another technical issue worth mentioning is the reliability of measures in platelet concentrates, which is strongly influenced by the type of instrument used and by the counting principle.

The CELL-DYN Sapphire uses two independent principles for measuring platelet counts: optical and electrical resistance (or impedance). These methods provide adequately concordant results both in the complete blood count from patients and in bags containing erythrocytes (WB, RCC and BC) but systematically discordant results in measurements on platelet concentrates (the result from optical counting is on average lower than the impedance method by 20-40%). In order to highlight the remarkable differences in the results obtained with the two systems (statistically significant, p<0.01), we report both the results obtained by means of optical counting (PLTo) and those by the impedance method (PLTi).

**Statistical analysis and evaluation criteria**

Statistical calculations were performed using formulae from Excel 2003 Professional (Microsoft Italia, Milan, Italy) and SPSS, release 21 (IBM Italia, Bologna, Italy).

For each variable the presence of distributive anomalies (trends and shape of the distribution) was pre-emptively looked for by visual inspection of frequency histograms and box-whisker plots. The Fisher's asymmetry coefficients and Pearson's kurtosis coefficients were also calculated. Applying the test of Grubbs (extreme Studentised deviate) we founded seven possible outliers (with p<0.01) in different series: since these measures are representative of the biological variability and there were no mistakes in the measurement (second analysis), all the extreme cases were included in the computing, according to an universally accepted criterion.

By means of the Kolmogorov-Smirnov non-parametric test, we checked that the observed samples effectively approximated a theoretical distribution of Gaussian type and calculated the confidence interval (CI) of the averages for p=0.05. After evaluating the homogeneity of the sample variances by means of Fisher's test, the statistical strength in the difference between the averages was verified with the Student's test for independent samples.

Although the t-test and F test are regarded as very robust, even when there are substantial differences from normality, we also evaluated the significance of difference between the medians by means of Wilcoxon's non-parametric test for independent samples for the series with outliers or with a ratio between asymmetry coefficients (or kurtosis) and the respective standard error higher than 2 (not reported in the tables), coming to conclusions which perfectly correspond to the preceding ones.

Lastly, we determined the number of bags for each blood component that did not comply with the product standard and, using a 2×2 contingency table and Fisher's exact test for small samples, we evaluated the probability of random difference between the control bags and the test bags.
Results

Table I presents the quality control data of WB, blood components and platelet pools obtained from the T&T control bags (average, standard deviation, confidence interval for p=0.05), compared with those of the T&B test bags. P values <0.05 in the t-test for the differences between averages were considered statistically significant and are reported.

Table II shows the number of bags, for each category, in which there was a deviation from the product standard. P values <0.05 in Fisher’s exact test were considered statistically significant and are reported.

In summary, the results obtained on blood components produced with the new T&B bags show that:

- the RCC appeared, on average, to be of better quality than the RCC obtained from traditional bags but, given the limited sample size, the differences found were clearly statistically significant only for the residual content in lymphocytes and platelets;
- the volume of plasma recovered was significantly greater (+14 mL for every FFP bag);
- for each platelet pool, on average, while the content in platelets was statistically higher (approximately +0.5 to +0.7×10¹¹, according to the counting method used), the final volume of residual plasma was 55 mL less. At the end of processing, each BC used contributed with around 31 mL vs 35 mL of control bags to the volume of the residual plasma;
- the quality controls performed on samples obtained from the new bags indicated that the blood components were of good quality and were more likely to reach European13,14 and national15,16 product standards than

Table I - Comparison of measurements made on control and test bags.

<table>
<thead>
<tr>
<th>T&amp;T T2466 control bags</th>
<th>T&amp;B PQ32150 test bags</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Comparison of measurements made on WB bags</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume (mL)</td>
<td>497 (8.86)</td>
<td>493-501</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>37.9 (2.51)</td>
<td>36.7-39.1</td>
</tr>
<tr>
<td>Red blood cells (mL)</td>
<td>189 (11.5)</td>
<td>184-194</td>
</tr>
<tr>
<td>Haemoglobin/unit (g)</td>
<td>65.9 (4.83)</td>
<td>63.6-68.2</td>
</tr>
<tr>
<td>Platelets/unit (×10¹¹)</td>
<td>1.04 (0.22)</td>
<td>0.94-1.14</td>
</tr>
<tr>
<td>White blood cells/unit (×10⁹)</td>
<td>2.63 (0.48)</td>
<td>2.41-2.85</td>
</tr>
<tr>
<td>Lymphocytes/unit (×10⁹)</td>
<td>0.96 (0.20)</td>
<td>0.87-1.05</td>
</tr>
<tr>
<td>Neutrophils/unit (×10⁹)</td>
<td>1.34 (0.31)</td>
<td>1.20-1.48</td>
</tr>
</tbody>
</table>

| **Comparison of measurements made on RCC bags** | | |
| Volume (mL) | 287 (13.0) | 281-293 | 294 (11.7) | 289-299 |
| Haematocrit (%) | 58.8 (2.5) | 57.6-60.0 | 59.8 (1.6) | 59.0-60.6 |
| Red blood cells (mL) | 169 (13.5) | 162-175 | 176 (11.4) | 171-181 |
| Red blood cell loss (mL) | 20 (6.7) | 17-23 | 14 (6.9) | 11-18 |
| Haemoglobin/unit (g) | 57.6 (5.1) | 55.2-60.0 | 60.2 (4.2) | 58.2-62.2 |
| Platelets/unit (×10¹⁰) | 16.0 (5.97) | 13.2-18.8 | 14¹ (1.39) | 1.49-2.79 p<0.001 |
| White blood cells/unit (×10⁹) | 0.94 (0.31) | 0.80-1.08 | 0.78 (0.26) | 0.66-0.90 |
| Lymphocytes/unit (×10⁹) | 0.13 (0.05) | 0.11-0.15 | 0.05b (0.06) | 0.02-0.08 p<0.001 |
| Neutrophils/unit (×10⁹) | 0.67 (0.26) | 0.55-0.79 | 0.63 (0.24) | 0.52-0.74 |

| **Comparison of plasma yield on FFP bags** | | |
| Volume (mL) | 245 (17.8) | 237-253 | 259 (12.5) | 253-265 p<0.05 |

| **Comparison of measurements made on platelet-pool bags** | | |
| Volume (mL) | 454 (15.5) | 447-461 | 394 (14.9) | 387-401 p<0.001 |
| PLTo/unit (×10¹¹) | 2.74 (0.58) | 2.47-3.01 | 3.21 (0.38) | 3.03-3.39 p<0.01 |
| PLTi/unit (×10¹¹) | 3.62 (0.67) | 3.31-3.93 | 4.29 (0.48) | 4.07-4.51 p<0.001 |
| Estimation of residual plasma (mL) | 209 (7.1) | 206-212 | 154 (5.8) | 151-157 p<0.001 |

(6 BC in each pool) (5 BC in each pool)

- 18 of 21 measures were under the instrumental limit of quantification. - 17 of 21 measures were under the instrumental limit of quantification.

T&T: Top & Top; T&B: Top & Bottom; SD: standard deviation; CI: confidence interval; WB: whole blood; RCC: red cell concentrate; FFP: fresh frozen plasma; PLTo: optical counting; PLTi: the impedance method; BC: buffy coat.
samples from conventional bags. This means that the standard operating procedures (centrifugation and blood components separation) were properly optimised.

Discussion

The average parameters (Table I) relative to the whole blood in mother bags did not differ significantly for total volume, haematocrit, red blood cell volume, haemoglobin or for total content of leucocytes, lymphocytes and neutrophils. These results suggest that the two types of bags are essentially equivalent with regards to pre-working values and allow us to attribute the differences detected in the products both to the innate characteristics of the mother bags and to different working conditions applied.

The statistical analysis did not reveal any substantial differences in the average amount of erythrocytes lost with BC and showed that the RCC produced with the two types of devices were similar concerning red cell parameters. Despite this, the total leucocyte content in the T&B bags was, on average, 16% lower and the average content of platelet and lymphocytes dropped dramatically (p<0.001), with many single measures being systematically lower in the T&B bags. This is consistent with previous studies comparing T&T and T&B bags, and allows us to attribute the differences detected in the products both to the typology of bag, the different value of ACE used comes into play (T&T bags) and to different working conditions applied.

The plasma volume produced with T&B bags was significantly greater (+5.7%, namely 14 mL for each unit), as was the average percent recovery: 84.3% vs 79.4%. In this case, besides the typology of bag, the different value of ACE used comes into play. In fact, although the most favourable conditions for maximum recovery of platelet-poor plasma are those obtained by working with an ACE between 70.0×10⁶ and 85.0×10⁶ (Figure 2), the application of a higher ACE to T&T mother bags, to squeeze more plasma, would significantly worsen the reduction of the leucocytes and platelets in the RCC bags, because of the increased adhesive force of the BC layer to the inner sides of the mother bag during centrifugation. This phenomenon, overlooked in literature, is visible in freshly centrifuged WB only if the bag is carefully inspected from the unlabelled side, without moving it. It consists of an off-white layer tenaciously adhering to the inner side, easily distinguishable from the proper buffy coat, which forms a flocculant separation line between red blood cells and plasma.

Through a semi-quantitative assessment made by external observation of a large number of T&T bags just centrifuged, we found that the adhesion of the off-white layer to the plastic material was tenacious and persistent. Indeed, not even the flow of red blood cells that rises to the top during the squeezing of the plasma has sufficient strength to remove it completely, leaving a highly variable portion of BC in the RCC bag (Figure 3). Increasing the ACE, increases the size and the adhesion of the off-white layer.

Regrettably, it is not possible to solve this problem by simply varying the steps of the work cycle of the T&T. In fact, in CompoMat® G4 the pressing area is divided, by a retractable moving blade, into an upper part dedicated to the isolation of BC (after squeezing the plasma), and a lower part dedicated to salvaging the erythrocytes. During

| Table II - Number of bags not complying with product standards, of 21 cases for each blood component. |
|--------------------------------------------------|---------------|----------------|----------------|----------------|
| **Variable** | **Product standard** | **N. of bags** | **N. of bags** | **p value** |
| WB | Volume (mL) | 405-495 | 0 | 0 |
| | Haemoglobin/unit (g) | >45 | 0 | 0 |
| RCC | Red blood cell loss (mL) | <30 | 1 | 0 |
| | Haematocrit (%) | 50-70 | 0 | 0 |
| | Haemoglobin/unit (g) | >43 | 0 | 0 |
| | White blood cells/unit (×10⁶) | <1.2 | 4 | 2 |
| | Platelets/unit (×10¹¹) | <20 | 4 | 0 |
| PLTo/unit (×10¹¹) | >2.5 (in 90% tested) | 9 | 0 | p<0.01 |
| PLTi/unit (×10¹¹) | 2 | 0 |

* Optical count of platelets; † Impedance count of platelets.
T&T: Top & Top; T&B: Top & Bottom; WB: whole blood; RCC: red cell concentrates; PLTo: optical counting; PLTi: the impedance method.
the separation cycle, the adherent layer remains in the lower chamber and cannot, therefore, be eliminated.

Because we could not improve our results simply by acting on the separation steps, we considered the possibility of reducing the BC adhesiveness by decreasing the overall force of the spin cycle. Our monitoring confirmed that in blood, centrifuged within a few hours of being collected, the visual evidence of the off-white layer gradually diminishes along with the ACE, down to almost disappearing at values lower than 30.0 × 10^6. Nevertheless, the average white blood cell contamination in the RCC bags does not decrease significantly while the variability of blood components increases, probably because the BC layer is not compact enough and tends to mix with the erythrocytes during the squeezing phase. At any rate, as shown in Figure 2, such weak centrifugation cannot be used in daily practice because it results in a plasma loss of about 10%, and the platelet contamination in the FFP might reach 10-15% of the native platelets.

Contrary to what occurs in the T&T bags, in the T&B bags the BC adhesiveness actually becomes an advantage because it happens right in the bag which will contain the separated buffy coat. This allows the use of an optimal ACE, achieving excellent packing of the red cells, a better yield of FFP with a minimal cellular contamination and BC richer in platelets. An increase of the ACE would not result in additional benefits and could compromise the integrity of the erythrocytes.

The divergent behaviour shown by the two types of bags with respect to adhesive properties of the BC is supported by the different association observed between the leucocyte content in the mother bag and in RCC bag, given optimised ACE both for T&T and T&B bags. For the T&T bags, each increase in the blood donor's leucocyte count produced a commensurate increase in the final RCC bag (r=0.71; p<0.001). When the content of the mother bag exceeds 3,000-3,500×10^9 leucocytes, which corresponds to a white blood cell count of 7,000-8,000/µL in the donor, the risk of obtaining non-compliant RCC is high.

For the T&B bags, on the other hand, there was not a statistically significant linear correlation (r=0.38; p>0.05) and a pseudo-asymptotic pattern was observed: the more white blood cells there were in the initial bag, the higher the percentage of cells which remained "trapped" in the BC (Figure 4).

With respect to the production of platelet pools, our results show that the quality of components obtained

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**Figure 2** - Fresh frozen plasma bag: plasma yield and platelet contamination as a function of ACE (accumulated centrifugal effect).

**Figure 3** - T&T (Top & Top) bag.
The arrow shows the layer adhering to the inner wall of the whole blood bag immediately after centrifugation (left) and after blood components separation (right). The liquid above the packed red blood cells is plasma and sodium chloride, adenine, glucose and mannitol, respectively.
Performance of Top & Bottom vs Top & Top bags was higher than that of components from T&T bags. In fact, in an adult non-splenectomised, this more concentrated product causes an average increase of 1,500-2,000 platelets/µL, as determined by a complete blood count, for each bag transfused. Moreover, given the reduced plasma volume, using products from T&B bags could also theoretically decrease plasma-mediated transfusion reactions and the risk of fluid overload.

Unfortunately, the method-dependence in counting platelets when these are isolated and concentrated, impedes the determination of exact correlations between the initial and final content, allowing only rough estimates of ultimate recovery.

The numbers of bags not complying with product standards are shown in Table II. All the WB bags complied in terms of volume donated and haemoglobin content. As far as concerns the residual content of platelets, all RCC obtained from test bags were well within the target, while four RCC of the control group exceeded the maximum limit. Only two RCC collected into the new bags, compared to four collected into the traditionally used bags, violated the target for total leucocytes. One T&T bag violated the target for the erythrocytes lost with the BC. From a statistical point of view, there were no significant differences for this group of data.

In contrast, with regards to platelet pools, nine traditional bags had a platelet content lower than the standard product according to optical counting and two of them highlighted the same problem even when using the most favourable impedance count. No platelet concentrate made with the new BC violated the target with the two counting systems. At Fisher’s exact test the difference was statistically significant only for the optical counts (p<0.01) and not for the impedance counts.

This contradictory result unequivocally confirms that the "state of the art" in counting the platelet content of the platelet concentrates for transfusion is still not satisfactory and that it is urgent for Blood Banks to adopt adequate and standardised analytical methods, even non-traditional ones if necessary. For this reason, the editors of several leading laboratory medicine journals have launched an official appeal for a full description of laboratory methods and specimen handling in clinical study reports, including laboratory tests not yet standardised. The same appeal could be addressed to the monitoring organisations of Transfusion Centres, such as the European Directorate for the Quality of Medicines & HealthCare (EDQM), to unequivocally declare the methods by which they define product standards. Even an External Quality Assessment (EQA) programme focused on blood components could be extremely useful to depict the current situation and to raise the awareness of users and manufacturers towards this important and underestimated problem.

Conclusion

The spontaneous adhesion of mononuclear cells to the plastic material of which blood bags are composed is a known, but only partially studied, phenomenon. In particular, we were unable to find studies that dealt specifically with haptotaxis of blood cells subjected to centrifugal forces.

In this study, we photographically documented and described in qualitative terms the systematic adhesion of a
part of the BC to the inner wall of the collection bag. The extent of this adhesion is directly proportional to the ACE.

Because of the construction geometry of the T&T bags, which keeps the RCC in the mother bag, the adherent layer is neither eliminated nor reduced significantly either by varying the working conditions of the automatic presses or by decreasing the centrifugal force. The only way to affect the adhesion is to use values of ACE so low as to make the bag-to-bag variability of all blood components unacceptable and to cause, for the FFP, insufficient recovery of volume with excessive cellular contamination.

This intrinsic limit of T&T bags led us to evaluate alternatives bags, such as the T&B bag. This bag contains a separation structure that is better suited to the mother bag, thereby allowing greater extraction of plasma and production of better quality BC and RCC.

We also considered the problem of cell counting in concentrated platelets, which has important implications for the definition and respect of product standards.

To conclude, substituting T&T bags with the new T&B bags could generate various benefits in blood component production:
- improved quality of RCC, with a substantial reduction in the content of lymphocytes and platelets, for better compliance with product standards;
- an increase in the amount of FFP sold to industry, with more revenue for our company;
- use of a smaller number of BC for each pool (at least 6 bags), decreasing the risk of infection in recipients;
- production of platelet pools much richer in platelets and containing less residual plasma, presumably with greater transfusion effectiveness and a decrease in plasma-mediated side effects;
- simplification of the production of the platelet pool using only one type of standardised BC, whether from the just tested PQ32150 or from the analogous CQ32250 with a soft pre-storage filter.

Moreover, considering that the BC from the bags tested are much richer in platelets than the traditional mini-BC, it would certainly be possible to use them to produce platelet-rich plasma from single BC of excellent quality, useful for both neonatal transfusions and for topical use (e.g. for the preparation of platelet gels).

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Authorship contributions
EC designed this research, performed the tests and the statistical evaluation, and drafted the manuscript. MN translated the manuscript and verified some technical aspects of the blood separation. EDB helped to verify the manuscript and revised some of the clinical aspects. PP verified some aspects regarding the quality control of the blood components. RB helped to draft and to revise the manuscript.

All Authors read and approved the final manuscript.

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The Authors certify that there is no conflict of interest with any financial organisation regarding the material discussed in the manuscript.

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