SUPPLEMENT ARTICLE

TRANSFUSION

Effect of leukoreduction and temperature on risk of bacterial growth in CPDA-1 whole blood: A study of *Escherichia coli*

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Abstract

Background: Collection of non-leukoreduced citrate-phosphate-dextrose-adenine (CPDA-1) whole blood is performed in walking blood banks. Blood collected under field conditions may have increased risk of bacterial contamination. This study was conducted to examine the effects of WBC reduction and storage temperature on growth of *Escherichia coli* (ATCC® 25922™) in CPDA-1 whole blood.

Methods: CPDA-1 whole blood of 450 ml from 10 group O donors was inoculated with *E. coli*. Two hours after inoculation, the test bags were leukoreduced with a platelet-sparing filter. The control bags remained unfiltered. Each whole blood bag was then split into three smaller bags for further storage at 2–6°C, 20–24°C, or 33–37°C. Bacterial growth was quantified immediately, 2 and 3 h after inoculation, on days 1, 3, 7, and 14 for all storage temperatures, and on days 21 and 35 for storage at 2–6°C.

Results: Whole blood was inoculated with a median of 19.5 (range 12.0–32.0) colony-forming units per ml (CFU/ml) *E. coli*. After leukoreduction, a median of 3.3 CFU/ml (range 0.0–33.3) *E. coli* remained. In the control arm, the WBCs phagocytized *E. coli* within 24 h at 20–24°C and 33–37°C in 9 of 10 bags. During storage at 2–6°C, a slow self-sterilization occurred over time with and without leukoreduction.

Conclusions: Storage at 20–24°C and 33–37°C for up to 24 h before leukoreduction reduces the risk of *E. coli*-contamination in CPDA-1 whole blood. Subsequent storage at 2–6°C will further reduce the growth of *E. coli*.

KEYWORDS
contamination, CPDA-1 whole blood, *E. coli*, leukoreduction, phagocytosis, storage, walking blood bank

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1 | INTRODUCTION

All blood cellular components, including whole blood, used in Norway are leukoreduced (LR) according to regulatory requirements. Whole blood is LR with a platelet-sparing filter. Local standard operating procedures allow for leukoreduction by filtration after rest at room temperature between 2 and 8 h after donation. The rational for the holding time is that during the rest period, the WBCs in whole blood are expected to phagocytize contaminating bacteria before removal from the blood by filtration. After filtration, whole blood is stored at 2–6°C until transfusion. Based on quality data regarding RBC storage, citrate-phosphate-dextrose-adenine (CPDA-1) could be a better storage solution because it allows for longer storage time.[1,2] However, a whole blood collection bag with CPDA-1 and a platelet-sparing filter is currently not commercially available.

Norwegian blood banks are hospital based. Blood is collected in GMP-approved blood donation facilities. In addition to serving the civilian hospitals, some blood banks support the Norwegian Armed Forces. As the supply chain may be challenging, it is necessary to establish safe systems for obtaining blood at site of injury. Under such circumstances, walking blood bank procedures covering the whole process from donor selection to transfusion have been implemented. Whole blood donated in a walking blood bank is intended for immediate use to maintain blood pressure and tissue oxygenation in the bleeding patient until surgical intervention is available.

Military protocols state that whole blood collected in a walking blood bank may be refrigerated within 8 h of donation as cold-stored whole blood if not used immediately. If this possibility is not available, whole blood may be kept at room temperature for up to 24 h.[3] However, pretested shipped whole blood is always the preferred solution.

The by far mostly applied method for leukoreduction in blood banks is removal by filtration. Based on hemovigilance data from many countries over decades, this is a safe procedure not increasing the risk of bacterial infections in the recipients.[4,5] Blood collected in a military walking blood bank is not LR.[6] When personnel, often with limited training, collect blood in a walking blood bank where hygiene procedures cannot be satisfactorily accomplished, there is a higher risk of bacterial contamination in the donated blood. Reducing the risk of bacterial contamination is therefore of importance.

In this study, we aimed to explore the effects of leukoreduction and storage temperature on bacterial growth in CPDA-1 whole blood. As a primary outcome, we investigated whether leukoreduction of whole blood protects against growth of E. coli during storage. As a secondary outcome, we examined whether storage at high temperatures leads to increased growth of E. coli and if such growth affects function and quality of CPDA-1 whole blood.

2 | MATERIALS AND METHODS

2.1 | Study design and ethics

This laboratory study was performed at the Department of Immunology and Transfusion Medicine at Haukeland University Hospital, Bergen, Norway, between September 2019 and March 2020. The regional committees for medical and health research ethics approved the study (REC id: 2017/157). All donors gave written informed consent to participate in the study before donation.

2.2 | Preparation of CPDA-1 whole blood

We collected 450 ml O RhD positive whole blood into bags with 63 ml CPDA-1 (PB-1CD456M5S, Terumo BCT) from 10 blood donors (four female and six male). Immediately after collection and baseline sampling, the whole blood was inoculated with 1 ml of a suspension containing 15,000 colony-forming units (CFU)/ml Escherichia coli (ATCC® 25,922™).

2.3 | Preparation of inoculum

Before each whole blood donation, we incubated one colony of E. coli in 25 ml Luria-Bertani medium at 200 rpm (311DS, Shaking Incubator, Labnet International Inc.) for 12–18 h at 37°C in normal atmosphere. To make a pellet of E. coli in exponential growth, the tube was centrifuged at 1900g (Kubota 8700, Kubota Corporation) in room temperature for 10 minutes. Parts of the pellet were resuspended in sterile saline (9 mg/ml NaCl, B. Braun) until OD_{600} (DEN-1B McFarland Densitometer, BioSan). The OD_{600}-concentration was determined to correspond to 0.75×10^8 CFU/ml E. coli by a pilot performed prior to investigation. A further dilution was made to achieve 15,000 CFU/ml. A 1 ml sample of the diluted E. coli was kept on ice until inoculation. To calculate the inoculation concentration and confirm the presence of E. coli in the inoculum, two positive controls were made as 10- and 100-fold dilutions of the inoculum. The positive controls were quantified as described below.

2.4 | Storage and sampling of CPDA-1 whole blood

After 2 h of rest at room temperature, the bags in the test arm ($n = 5$) were LR with a platelet-sparing filter (Imuflex WB-SP, BB*LGQ456E6, Terumo BCT), whereas bags in the control arm ($n = 5$) remained unfiltered.

After 3 h, the bags were gently mixed and transferred to three final storage bags (Teruflex, BB*T015CM, *Terumo BCT*), which were kept at 2–6°C (4°C) for 35 days and at 20–24°C (22°C) or 33–37°C (35°C) for 14 days. The bags were sampled by sterile welding (TSCD II Sterile Tubing Welder, *Terumo BCT*) of a Teruflex bag to the storage bags. The bags were gently mixed before an appropriate volume was removed.

The sampling time points for bacterial growth were as follows: Days 1, 3, 7, and 14 for all storage temperatures and additionally on days 21 and 35 for bags stored at 4°C. Samples for hematology and blood gas were analyzed at all sample time points for bags stored at 4°C, while only until day 7 for bags stored at 22°C and 35°C. Samples for thromboelastography were analyzed immediately and 3 h after donation, on day 7 for all storage temperatures and additionally on day 35 for bags stored at 4°C.

2.5 | Laboratory investigations

To ensure that there were no complications from other bacteria already present in the whole blood, we performed bacterial controls immediately after donation and before inoculation with *E. coli*. A three-way stopcock (Discofix® C, *B. Braun Melsungen AG*) was sterile-welded to the whole blood bag and 10 ml whole blood was transferred aseptically to aerobic (BacT/ALERT FA Plus, *bioMérieux SA*) and anaerobic (BacT/ALERT FN Plus, *bioMérieux SA*) culture bottles, which were incubated at 36°C for 7 days (BacT/ALERT® 3D 60, *bioMérieux SA*).

Quantification of *E. coli* was performed by pipetting and dispersing 100 μl of whole blood onto blood agar plates in triplicate. Where appropriate based on previous results, clots, and odor, we diluted the samples in 10-fold increments with sterile saline and dispersed at least three dilution steps. The blood agar plates were incubated at 37°C for 18–24 h. Plates with <200 colonies were counted. The mean of three plates was used to calculate CFU/ml *E. coli* in whole blood.

Residual WBC (rWBC) was counted by flow cytometry (BD Leucocount Kit/BD FACSCanto II, *BD Biosciences*) after leukoreduction of the test bags. Hematology and blood gas parameters were measured at all sample points, except day 14 for bags stored at 22°C and 35°C. If visible clots were detected, these tests were not analyzed because the clots would block capillaries in the instruments. Hemoglobin (HBG), hematocrit (HCT), platelet count (PLT), and WBC count were analyzed in K_2EDTA on a hematology analyzer (Cell-Dyn Sapphire, *Abbott Diagnostics*). Plasma-hemoglobin (plasma-HGB) was measured using a photometer (HemoCue Plasma/Low Hb, *HemoCue AB*). pH, pO_2, pCO_2, potassium, glucose,

and lactate were measured on a blood gas analyzer (ABL825 FLEX, *Radiometer Medical ApS*).

Viscoelastic hemostatic properties were measured by thromboelastography (TEG 5000, *Haemonetics Corporation*) immediately after donation, at 3 h and on day 7. Bags stored at 4°C were additionally measured on day 35. Briefly, 1 ml of whole blood was activated with 40 μl of kaolin, of which 340 μl was transferred to a plain cup containing 20 μl of 0.2 M $CaCl_2$ to overturn the effect of citrate. The analysis ran until 30 minutes after reaching MA. The parameters recorded from TEG analysis were as follows: Time to first clot formation (*R*), time from *R* until the clot reached 20 mm (*K*), rate of clot formation (*α*), and maximum clot strength (MA).

2.6 | Visual inspection

From day 1, we performed a visual inspection of the blood bags to investigate if there were any visual changes occurring that we could use as markers of bacterial growth. Clotting and the color of plasma and whole blood were evaluated.

2.7 | Statistical analysis

SPSS (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.) was used to create the database and perform statistical analyses. Due to the low number of whole blood bags in this study, we only performed descriptive analyses, which are presented as median (min–max) for bacterial concentration and mean ± SD for hematology, blood gas, and functional analyses.

3 | RESULTS

CPDA-1 whole blood of 450 ml from 10 group O blood donors was inoculated with a median of 19.5 (12.0–32.0) CFU/ml *E. coli*. Samples taken immediately after inoculation of *E. coli* confirmed recovery of the inoculated *E. coli* in all bags, with 10.0 (3.3–26.7) CFU/ml.

3.1 | Effect of leukoreduction on growth of *E. coli*

After 2 h of rest at room temperature, *E. coli* was still detectable in 9 of 10 bags with 13.5 (0.0–23.3) CFU/ml. Five bags in the test arm were then LR through a platelet-sparing filter. LR did not remove *E. coli* from the whole blood (Table 1). When measured 3 h after

TABLE 1 Growth of *E. coli* in CPDA-1 whole blood on day of donation

	Positive control[a]	Immediately after inoculation	Two-hour rest	Three-hour rest or filtration
Non-leukoreduced control arm (CFU/ml)	20.9	26.7	23.3	10.0
	18.6	3.3	20.0	10.0
	12.0	10.0	20.0	3.3
	23.2	20.0	16.7	26.7
	32.0	10.0	6.7	16.7
Median (min–max)	20.9 (12.0–32.0)	10.0 (3.3–26.7)	20.0 (6.7–23.3)	10.0 (3.3–10.0)
Leukoreduced test arm (CFU/ml)	23.3	6.7	3.3	3.3
	15.7	6.7	10.0	10.0
	18.0	10.0	23.3	33.3
	19.9	13.3	0.0	0.0
	19.0	6.7	6.7	0.0
Median (min–max)	19.0 (15.7–23.3)	6.7 (6.7–13.3)	6.7 (0.0–23.3)	3.3 (0.0–33.3)

Abbreviation: CFU/ml, colony forming units/ml.

[a]A 10-fold dilution of the inoculum was measured to calculate inoculation concentration.

inoculation, the LR and the non-leukoreduced (nLR) arm had a concentration of 3.3 (0.0–33.3) CFU/ml and 10.0 (3.3–26.7) CFU/ml, respectively (Table 1). We found 0.0 CFU/ml *E. coli* in one bag after 2 h rest and in two bags immediately after filtration. The bags were not sterile, as we found *E. coli* at later sample points. After leukoreduction, we detected rWBC concentration of mean $0.09 \pm 0.13 \times 10^6$/unit in four bags in the test arm. A pre-analytical error occurred before analysis of the fifth tube. Due to storage at room temperature overnight, *E. coli* grew in the test tube in a proportion to give a measuring error.

3.2 | Effect of storage temperature on growth of *E. coli*

Refrigerated storage at 4°C inhibited the growth of *E. coli* in both arms. All bags stored at 4°C had detectable growth at least one of the first 7 days, whereas none of the bags had detectable growth on days 14, 21, and 35 (Table 2).

The bags in the LR arm stored at 22°C had a median of 3.3×10^4 (2.0×10^3–8.0×10^5) CFU/ml *E. coli* from day 1, reaching 6.7×10^8 (4.0×10^8–7.0×10^8) CFU/ml on day 14 (Table 2). In the nLR arm, only one bag had detectable growth on day 1, reaching 2.5×10^8 CFU/ml on day 14. Four of the five bags in the control arm had detectable growth on day 14 (Table 2). One bag had visible clots on day 14 but no detectable growth in a $1:10^6$ dilution.

Three of five bags in the LR arm stored at 35°C had bacterial growth from day 1, whereas two bags had no detectable growth throughout storage for up to 14 days (Table 2). No nLR bags stored at 35°C had detectable growth on days 1 and 3. There was, however, detectable growth from day 7 in two of five bags. One of the bags with detectable growth on day 7 was completely coagulated on day 14, which made sampling impossible. One bag had visible clots on day 14, but no detectable growth with a dilution of $1:10^3$.

3.3 | Visual inspection

All bags with exponential growth of *E. coli* provided visible clots from day 3 onwards, contrasting no visible clots on day 1. One bag in the control arm had no visible clots despite an *E. coli* concentration of 7.0×10^5 CFU/ml on day 14. This bag had no detectable growth on day 7.

The storage bags used in this study were gas permeable, and we found that RBCs in bags stored at 4°C became more and more vibrantly red, which coincided with an increase in pO_2 levels from mean 7.3 ± 1.4 kPa on day 1 to 34.1 ± 1.1 kPa on day 35. There was no relation between darkening of color in whole blood stored at 22°C and 35°C and bacterial growth. Visual inspection of the bags before mixing did not always show hemolysis that had occurred since the last sampling. In seven bags with mean plasma-HGB of 0.47 g/L (range 0.4–0.6) at the previous sample-point and no visible hemolysis before mixing, we found a mean of 5.71 g/L (range 2.2–15.1) plasma-HGB, which would result in hemolysis above 0.8%.

TABLE 2 Growth of *E. coli* in CPDA-1 whole blood during storage at 4°C, 22°C, and 35°C

Storage at 4°C	Day 1	Day 3	Day 7	Day 14	Day 21	Day 35
Non-leukoreduced control arm (CFU/ml)	66.7	30.0	6.7	0.0	0.0	0.0
	0.0	3.3	0.0	0.0	0.0	0.0
	50.0	16.7	3.3	0.0	0.0	0.0
	43.3	6.7	6.7	0.0	0.0	0.0
	26.7	20.0	20.0	0.0	0.0	0.0
Median (min–max)	43.3 (0.0–66.7)	16.7 (3.3–30.0)	6.7 (0.0–20.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
Leukoreduced test arm (CFU/ml)	0.0	16.7	6.7	0.0	0.0	0.0
	3.3	3.3	0.0	0.0	0.0	0.0
	23.3	20.0	3.3	0.0	0.0	0.0
	0.0	13.3	0.0	0.0	0.0	0.0
	16.7	0.0	3.3	0.0	0.0	0.0
Median (min–max)	3.3 (0.0–23.3)	13.3 (0.0–20.0)	3.3 (0.0–6.7)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)

Storage at 22°C	Day 1	Day 3	Day 7	Day 14		
Non-leukoreduced control arm (CFU/ml)	0.0^a	0.0	0.0	3.6×10^7		
	0.0	0.0	0.0	$2.0 \times 10^{5\ b}$		
	0.0	0.0	0.0	0.0^c		
	0.0	0.0	0.0	7.0×10^5		
	1.4×10^3	5.0×10^6	4.4×10^7	2.5×10^8		
Median (min–max)	$0.0\ (0.0–1.4 \times 10^3$	$0.0\ (0.0–5.0 \times 10^6)$	$0.0\ (0.0–4.4 \times 10^7)$	7.0×10^5 $(0.0–2.5 \times 10^8)$		
Leukoreduced test arm (CFU/ml)	$2.0 \times 10^{3\ b}$	1.4×10^8	2.3×10^8	6.4×10^8		
	3.3×10^4	3.9×10^7	1.9×10^8	6.8×10^8		
	8.0×10^5	3.6×10^6	1.1×10^8	4.0×10^8		
	1.9×10^4	3.3×10^6	1.8×10^8	6.7×10^8		
	2.0×10^5	2.7×10^7	1.9×10^8	7.0×10^8		
	3.3×10^4	2.7×10^7	1.9×10^8	6.7×10^8		
Median (min–max)	$(2.0 \times 10^3–8.0 \times 10^5)$	$(3.3 \times 10^6–1.4 \times 10^8)$	$(1.1 \times 10^8–2.3 \times 10^8)$	$(4.0 \times 10^8–7.0 \times 10^8)$		

Storage at 35°C	Day 1	Day 3	Day 7	Day 14		
Non-leukoreduced control arm (CFU/ml)	0.0^a	0.0	0.0	0.0^c		
	0.0	0.0	0.0	0.0		
	0.0	0.0	$2.0 \times 10^{3\ b}$	NA		
	0.0	0.0	0.0	0.0		
	0.0	0.0	1.8×10^4	1.2×10^9		
Median (min–max)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	$0.0\ (0.0–1.8 \times 10^4)$	$0.0\ (0.0–1.2 \times 10^9)$		
Leukoreduced test arm (CFU/ml)	$2.0 \times 10^{3\ b}$	4.5×10^8	8.7×10^8	6.3×10^8		
	1.0×10^4	9.7×10^7	6.2×10^8	3.5×10^8		
	0.0^a	0.0	0.0	0.0		
	0.0	0.0	0.0	0.0		
	2.4×10^7	4.3×10^8	7.8×10^8	6.2×10^8		
	2.0×10^3	9.7×10^7	6.2×10^8	3.5×10^8		

TABLE 2 (Continued)

Storage at 4°C	Day 1	Day 3	Day 7	Day 14	Day 21	Day 35
Median (min–max)	$(0.0–2.4 \times 10^7)$	$(0.0–4.5 \times 10^8)$	$(0.0–8.7 \times 10^8)$	$(0.0–6.3 \times 10^8)$		

[a]Three bags had no detectable growth in the dilutions used at that sample point.

[b]Four bags had >200 colonies on the blood-agar plates with the dilutions used. We were unable to estimate the correct concentration of bacteria in these samples.

[c]Two bags had no detectable growth in the dilutions used. However, due to clots in the bags we suspect false negative results.

Abbreviation: CFU/ml, Colony forming units/ml.

Five bags stored at 35°C, three in the LR arm and two in the nLR arm, had visible air bubbles in the bag on day 3. The three bags in the test arm had extensive growth, whereas the two bags in the control arm had no detectable bacterial growth.

There was a distinct smell from bags with bacterial growth, which corresponded to the extent of bacterial growth.

3.4 | The effects of *E. coli* on functional and quality control markers

Samples for functional test and quality control analysis were taken on day 1 from all bags, but from day three, all bags with exponential growth of *E. coli* had clots of differing sizes, which made analysis in automated analyzers impossible. Only bacterial quantification and plasma-HGB were performed on the clotted samples. Table 3 shows hematology and blood gas results, while Table 4 shows thromboelastography results from the bags stored at 4°C. No bags had hemolysis >0.8% at end of storage (Table 3). However, there was a decline in platelet count during storage.

4 | DISCUSSION

Escherichia coli (ATCC® 25,922™) is a strain of bacteria used in many laboratory experiments.[7–11] It has a rapid growth rate, activates but is not killed by complement, does not produce verotoxin, and is coagulase negative.[12] Additionally, *E. coli* has been implemented in severe septic transfusion reactions, including fatalities.[13,14] The bacterium enabled us to examine bacterial growth in CPDA-1 whole blood with and without WBCs, because *E. coli*, as observed in our data, does not absorb to the filter during leukoreduction. We used a medium-sized inoculum to ensure detection in 100 μl whole blood immediately after inoculation and thereby confirm a successful inoculation. This inoculum does not simulate contamination from an unsuccessful disinfection of skin, but may be representative of bacteremia or sepsis.[15]

4.1 | Effect of leukoreduction on growth of *E. coli*

Our results indicate that a longer resting period at 22°C would facilitate phagocytosis[16] and thereby enhance sterility as the phagocytized bacteria are removed during filtration.[17] Sanz et al. showed that prolonged storage of whole blood at room temperature for 16 h yielded fewer *E. coli* than whole blood held for 6 h,[18] which is supported by our results. Moroff et al. showed that in vivo survival in platelets stored for 5 days and RBCs stored for 35 days was similar when whole blood was held at room temperature for 8 or 24 h before component preparation,[19] which indicates that prolonged storage of whole blood at room temperature before cold storage is possible.

Lack of growth combined with the slow self-sterilization in both study arms indicates that WBCs have no influence on bacterial growth at 4°C. Our findings indicate that, in field conditions, it could be advantageous to store whole blood nLR after a prolonged hold at room temperature. However, further studies should be performed to evaluate the effects of prolonged storage at room temperature before cold storage and/or leukoreduction for a broader spectrum of bacteria.

4.2 | Effect of storage temperature on growth of *E. coli*

The phenomenon with auto-sterilization that we observed during storage at 4°C has been described previously for RBCs contaminated with *E. coli*[9,20] as well as a bacteriostatic effect in platelet concentrates stored at 4°C for 3 and 5 days.[11,21] Braude et al. found that only some strains of *E. coli* survived in citrated blood stored at 4–8°C for 20 days.[22]

TABLE 3 Results of blood gas and hematology analyses on whole blood stored at 4°C from day 1 to end of storage day 35

Analysis[a]	Arm	Day 1	Day 3	Day 7	Day 14	Day 21	Day 35
pH ()	Control	7.16 ± 0.01	7.11 ± 0.02	7.02 ± 0.03	6.92 ± 0.04	6.84 ± 0.04	6.70 ± 0.05
	Test	7.16 ± 0.02	7.12 ± 0.02	7.05 ± 0.03	6.95 ± 0.03	6.88 ± 0.03	6.75 ± 0.03
pCO_2 (kPa)	Control	10.2 ± 0.6	10.8 ± 0.8	12.0 ± 0.8	11.9 ± 1.1	10.8 ± 0.6	7.7 ± 0.6
	Test	10.2 ± 0.4	10.8 ± 0.4	11.3 ± 0.2	11.5 ± 40.3	10.5 ± 0.6	7.9 ± 0.6
pO_2 (kPa)	Control	8.1 ± 1.4	10.5 ± 3.1	15.7 ± 47.3	29.0 ± 3.4	32.6 ± 0.6	34.1 ± 1.2
	Test	6.6 ± 1.0	7.5 ± 0.4	10.5 ± 0.6	27.4 ± 2.4	32.2 ± 1.1	34.1 ± 1.1
Potassium (mmol/L)	Control	4.3 ± 0.3	6.4 ± 0.8	9.6 ± 1.4	14.1 ± 2.0	17.6 ± 2.4	22.6 ± 1.7[b]
	Test	4.2 ± 0.3	6.5 ± 0.5	9.9 ± 1.0	14.2 ± 1.7	17.7 ± 1.8	22.5 ± 1.7[b]
Glucose (mmol/L)	Control	24.1 ± 0.3	23.3 ± 1.0	21.5 ± 0.9	18.8 ± 1.0	16.1 ± 1.1	11.5 ± 1.0
	Test	24.5 ± 1.0	23.5 ± 1.1	22.2 ± 01.1	19.3 ± 1.2	16.9 ± 0.8	12.4 ± 0.5
Lactate (mmol/L)	Control	3.2 ± 0.3	5.1 ± 0.5	8.4 ± 0.8	13.6 ± 1.2	18.2 ± 2.0	25.6 ± 3.8
	Test	3.8 ± 0.7	5.4 ± 0.9	8.4 ± 1.3	13.1 ± 70.9	17.6 ± 1.8	24.4 ± 1.1
Hemoglobin (g/dl)	Control	12.2 ± 1.1	12.2 ± 1.1	12.1 ± 1.0	12.2 ± 1.0	12.3 ± 1.0	12.2 ± 1.0
	Test	12.3 ± 0.9	12.3 ± 0.9	12.3 ± 0.8	12.4 ± 0.9	12.3 ± 0.9	12.3 ± 0.8
Hematocrit (1)	Control	0.38 ± 0.03	0.38 ± 0.03	0.39 ± 0.03	0.40 ± 0.04	0.39 ± 0.03	0.40 ± 0.03
	Test	0.39 ± 0.03	0.38 ± 0.03	0.39 ± 0.03	0.39 ± 0.02	0.39 ± 0.03	0.40 ± 0.02
Plasma-Hemoglobin (g/L)	Control	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	0.6 ± 0.3	0.7 ± 0.4
	Test	0.3 ± 0.1	0.3 ± 10.1	0.4 ± 0.1	0.4 ± 0.0	0.5 ± 0.1	0.7 ± 0.3
Hemolysis (%)	Control	0.15 ± 0.03	0.17 ± 0.03	0.18 ± 0.04	0.26 ± 0.07	0.29 ± 0.13	0.32 ± 0.17
	Test	0.15 ± 0.03	0.15 ± 0.03	0.20 ± 0.03	0.21 ± 0.03	0.24 ± 0.06	0.33 ± 0.17
Platelet count ($\times 10^9$/L)	Control	157 ± 29	159 ± 27	155 ± 33	123 ± 31	122 ± 33	115 ± 42
	Test	143 ± 46	138 ± 53	132 ± 46	117 ± 46	93 ± 33	72 ± 38
White blood cell count ($\times 10^9$/L)	Control	4.4 ± 1.1	4.4 ± 1.1	4.3 ± 1.3	3.9 ± 1.4	3.4 ± 1.4	2.9 ± 1.2

[a]Mean ± SD.
[b]$n = 4$.

Analysis[a]	Sample point / Temperature	Day 0_0 / RT	Day 0_3[b] / RT	Day 7 / 4°C	Day 35 / 4°C
TEG R (min)	Control	6.7 ± 0.9	7.8 ± 1.2	7.6 ± 1.0	7.0 ± 0.9
	Test	6.5 ± 0.9	9.6 ± 1.3	7.3 ± 0.4	6.5 ± 0.8
TEG K (min)	Control	1.6 ± 0.3	2.0 ± 0.3	2.1 ± 0.3	4.6 ± 0.7
	Test	1.7 ± 0.3	2.5 ± 0.7	2.3 ± 0.4	5.7 ± 1.5
TEG α (°)	Control	66.7 ± 4.0	62.0 ± 4.0	61.2 ± 3.1	49.7 ± 4.0
	Test	65.4 ± 3.8	55.5 ± 7.4	58.3 ± 3.7	47.7 ± 9.5
TEG MA (mm)	Control	63.4 ± 3.5	61.9 ± 2.5	61.2 ± 3.1	42.5 ± 3.7
	Test	64.8 ± 1.9	57.2 ± 4.6	58.3 ± 3.8	42.5 ± 5.6

TABLE 4 Viscoelastic hemostatic properties measured by thromboelastography

Note: RT, room temperature; 4°C, storage at 2–6°C; R, time to first clot formation; K, time from R until the clot reached 20 mm; α, rate of clot formation, and MA, maximum clot strength.
[a]Mean ± SD.
[b]After leukoreduction of bags in the test arm.

The delay in bacterial growth in nLR whole blood stored at 22°C or 35°C is in accordance with previous literature reporting that WBCs can phagocytize *E. coli* in whole blood at 22°C and 35°C.[23] However, the phagocytosis is temperature-dependent,[24] and our results suggest that the phagocytosis is better preserved at 35°C as

compared with 22°C and 4°C. At 35°C, in both arms, two of five bags did not have detectable growth during storage. However, prolonged storage at 35°C is not advisable because of the reduced quality of whole blood (Table S1).

We found that nLR whole blood was able to phagocytize *E. coli* in four of five bags and keep the bacteria undetectable for a period. After the WBCs disintegrated, bacteria were released into the whole blood, as described by Högman et al.[16] One nLR bag stored at 22°C, in which bacterial growth was demonstrated already on storage day 1, had a lower WBC count 3.2×10^9/L, than the other four bags (range $3.7–6.3 \times 10^9$/L). As we do not have data on different WBC subsets, we can only speculate that the whole blood of some donors may be incapable of executing phagocytosis at a sufficient level to remove the concentration of bacteria we used.[25]

4.3 | Visual inspection

Visual inspection of all blood components before release is an important part of a quality control system.[26] Bacterial contamination may lead to discoloration and hemolysis, which may be detectable by visual inspection.[27] Sealed segments of tubing attached to the bag may be used to assess the changes in color.[28] When whole blood bags are stored upright, RBCs sediment, and the supernatant should, under ideal conditions, be a clear yellow. A way of detecting hemolysis in stored whole blood is to turn the bags over daily to make sedimentation better reveal the true color of plasma before issuing.[28,29]

Clot formation is known to accompany bacterial contamination of RBC concentrates.[30] A result of bacterial growth in our study was extensive coagulation of the whole blood.[31–33] There was, however, a window period where bacterial growth was detected by testing without any visual signs. One bag without any visual signs of bacterial growth had 2.4×10^7 CFU/ml *E. coli*, which is associated with serious transfusion reactions and possible fatality.[34,35]

4.4 | The effects of *E. coli* on functional and quality control markers

Statistical comparative analyses were not performed due to low number of bags. The LR arm had fewer platelets at the end of storage, which may have started at filtration, where we found a 10% (range 4–21) loss. Sivertsen et al. found similar results in cold-stored leukoreduced CPDA-1 whole blood.[2] Pidcoke et al. found that cold storage better preserved hemostatic function in CPD-whole blood than room temperature during storage for

21 days.[36] As shown in Table 4, we found that cold storage preserved hemostatic function until day 35. Meledeo et al. compared three different storage solutions for nLR whole blood.[37] They found similar hematology results as we did in nLR CPDA-1 whole blood stored cold for 35 days.

4.5 | Limitations of our study

The sampling volume of 100 μl whole blood in triplicate for bacterial quantification has its limits. However, it is common practice.[38,39] In bags with low concentration of bacteria, there is a possibility of false negative results. In our study, two bags with detectable bacteria immediately after inoculation but no detectable bacteria 3 h after inoculation had detectable *E. coli* at a later time-point, which confirm false negative results in bags with few colony-forming units.

We could only collect samples for laboratory investigation from whole blood without clots. Since five LR bags stored at 22°C and three LR bags stored at 35°C developed clots, we missed almost all samples after day 1 in the test arm.

5 | CONCLUSIONS

LR by filtration 2 h after donation did not protect whole blood from growth of *E. coli*, because the bacteria passed the filter simultaneously as bactericidal protection from WBCs was removed. There was less growth of *E. coli* in nLR whole blood on days 1 and 3 when stored at 22°C and 35°C. During storage at 4°C, a slow self-sterilization occurred. Our findings indicate that postponed filtration will better protect against bacterial contamination. We found more platelets and no reduced quality in nLR whole blood stored at 4°C compared with LR. Further studies should be performed to find the ideal time to transfer whole blood into cold storage.

CONFLICT OF INTEREST

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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