

Temperature Intelligence®



Bacterial Growth in Leukoreduced RBCs During and Following a 3 hr Temperature Excursion American Red Cross
Wagner SJ, Hapip CA, Abel LA, American Red Cross Holland Lab, Rockville, MD 20855

Background
It is not uncommon that power interruptions occur at blood centers and hospitals during extreme weather events. When backup generators fail, refrigerated blood units beyond 6°C. Previous studies have been conducted which evaluate bacterial growth during 30 minutes (the thirty minute rule) or just prior to transfusion (the 4 hour rule)^{1,2} However, no study has been conducted directly comparing bacterial growth in leukoreduced RBCs stored with continuous cold storage compared to RBCs stored with a temperature excursion. This study evaluates the levels of several bacteria, some of which are psychophiles, in refrigerated leukoreduced RBCs during and following a 3-hour temperature excursion towards room temperature.

References:
1. Dandekar 2011, 53:80-9
2. Van Dong 2012:301-306

Experimental Design and Methods
Two ABO, Rh identical leukoreduced RBCs were prepared from whole blood collected from consenting donors (Final Round Label). The units were pooled, mixed and inoculated on Day 7 with 10⁶ CFU/ml (for quantitative plate assay) of *P. fluorescens*, *Y. enterocolitica*, *S. marcescens*, *S. liquefaciens*, *E. coli*, *S. aureus*, *E. cloacae* and *S. caprore*. The pool was divided equally into two units and stored at 4°C. On Day 14 one of the two units (TEST) was placed on the laboratory bench (20-24°C) for 3 hours, surface temperature recorded, and then returned to 4°C storage. The other unit was maintained in refrigerated storage (control). Samples were taken for quantitative plate assay on Day 7, Day 14 pre-excision (pre), Day 14 post-excision (post), Day 28 and Day 42. Experiments were repeated 3 times per organism.

Results
At the end of the 3-hour temperature excursion, the surface temperature of units were 18.7 ± 0.8°C. Bacterial results are expressed as (CFU/ml_{post})/(CFU/ml_{control}) in the table below. No significant differences were observed between the growth of test and control units during storage for *P. fluorescens*, *E. coli*, *S. aureus*, *E. cloacae* or *S. caprore*. On Day 14 post excursion, the bacterial count in test units containing *Y. enterocolitica* declined approximately 4-fold relative to control units; no differences were observed in other temperatures. For *S. marcescens*, significantly greater levels of bacteria (2- and 3-4 fold) were observed in test units relative to those of controls on Days 28 and 42, respectively. Bacteria counts of *S. liquefaciens* that were subjected to the temperature excursion were 4-4 fold greater than those post-excision of control units on Day 14, but this difference did not attain statistical significance.

Organism	Day 7	Day 14 pre	Day 14 post	Day 28	Day 42
<i>P. fluorescens</i>	1.0	0.76 ± 0.49	0.95 ± 0.53	1.03 ± 0.50	0.86 ± 0.34
<i>Y. enterocolitica</i>	1.0	0.75 ± 0.31	0.05 ± 0.03	0.81 ± 0.01	0.76 ± 0.31
<i>S. marcescens</i>	1.0	1.97 ± 0.29	1.69 ± 0.43	3.19 ± 0.93	5.27 ± 0.43
<i>S. liquefaciens</i>	1.0	1.42 ± 0.43	4.28 ± 2.28	1.32 ± 0.36	1.93 ± 0.32
<i>E. coli</i>	1.0	0.94 ± 0.46	1.43 ± 0.43	No growth	No growth
<i>S. aureus</i>	1.0	1.51 ± 0.40	1.06 ± 0.37	0.83 ± 0.31	1.78 ± 0.39
<i>E. cloacae</i>	1.0	0.90 ± 0.43	1.39 ± 0.53	No growth	No growth
<i>S. caprore</i>	1.0	0.53 ± 0.37	0.39 ± 0.35	No growth	No growth

Conclusions
A 3-hour room temperature excursion in RBCs did not affect bacterial counts in *P. fluorescens*, *E. coli*, *S. aureus*, *E. cloacae*, and *S. caprore*, temporarily decreased the levels of *Y. enterocolitica* immediately following temperature excursion but enhanced the growth of *S. marcescens* during later storage relative to continuous 4-6° storage.

Poster Presentation by American Red Cross at AABB 2018¹ Learning More About Bacterial Growth in RBCs After Room Temperature Exposure

At the recent AABB Annual Meeting in Boston, a poster presented by the American Red Cross caught the attention of many attendees— including our team. Since our work with blood banks is focused on helping monitor the temperature of blood products during transport and temporary storage, we're always excited to find solid research by the experts.

In essence, the Red Cross' poster shared the results of testing they'd conducted to determine if there was significant bacterial growth in RBCs after a 3-hour temperature excursion towards room temperature. Of the six bacteria studies,

the results indicated that such a temperature exposure enhanced the growth of *S. marcescens* during later storage.

This is a critical finding for blood banks on two levels. First, *S. marcescens* is often involved in HAIs (Hospital Acquired Infections). Second, blood banks no longer have control over blood products once they have been issued, making it particularly important to have measures in place to ensure that the appropriate 6°C storage and 10°C transport temperatures have been maintained. We hope that you find this information helpful.

¹Publication shared with permission of the American Red Cross.

Background/Case Studies

It is not uncommon that power interruptions occur at blood centers and hospitals during extreme weather events. When backup generators fail, refrigerated blood warms beyond 6°C. This study evaluates the levels of several bacteria in refrigerated leukodepleted RBCs during and following a 3- hour temperature excursion towards room temperature.

Study Design/Method

Two ABO, Rh identical leukoreduced RBCs were prepared from whole blood collected from consenting donors (Fenwal, Round Lake). The

units were pooled, mixed and inoculated on Day 7 with 10-100 CFU/mL (for quantitative plate assay) of *P. fluorescens*, *Y. enterocolita*, *S. marcescens*, *S. liquefaciens*, *E. coli* and *S. aureus*. The pool was divided equally into two units and stored at 1-6°C. On Day 14 one of the two units (TEST) was placed on the laboratory bench (20-24°C) for 3 hours, surface temperature recorded, and then returned to 1-6°C storage. The other unit was maintained in refrigerated storage (control). Samples were taken for quantitative plate assay on Day 7, Day 14 pre-excursion (pre), Day 14 post-excursion (post), Day 28 and Day 42. Experiments were repeated 3 times per organism.

Bacterial Growth in Leukoreduced RBCs During and Following a 3 hr Temperature Excursion



American Red Cross

Wagner SJ, Hapip CA, Abel LA, American Red Cross Holland Lab, Rockville, MD 20855

Background

It is not uncommon that power interruptions occur at blood centers and hospitals during extreme weather events. When backup generators fail, refrigerated blood warms beyond 6°C. Previous studies have been conducted which evaluate bacterial growth during 30 minutes (the thirty minute rule) or just prior to transfusion (the 4 hour rule).^{1,2} However, no study has been conducted directly comparing bacterial growth in leukoreduced RBCs stored with continuous cold storage compared to RBCs stored with a temperature excursion. This study evaluates the levels of several bacteria, some of which are psychrophiles, in refrigerated leukodepleted RBCs during and following a 3-hour temperature excursion towards room temperature.

References:

1. Transfusion 2013; 53:851-9
2. Vox Sang 2013;105:100-7

Experimental Design and Methods

Two ABO, Rh identical leukoreduced RBCs were prepared from whole blood collected from consenting donors (Fenwal, Round Lake). The units were pooled, mixed and inoculated on Day 7 with 10-100 CFU/mL (for quantitative plate assay) of *P. fluorescens*, *Y. enterocolita*, *S. marcescens*, *S. liquefaciens*, *E. coli* *S. aureus*, *E. cloacae* and *S caprae*. The pool was divided equally into two units and stored at 1-6°C. On Day 14 one of the two units (TEST) was placed on the laboratory bench (20-24°C) for 3 hours, surface temperature recorded, and then returned to 1-6°C storage. The other unit was maintained in refrigerated storage (control). Samples were taken for quantitative plate assay on Day 7, Day 14 pre-excursion (pre), Day 14 post-excursion (post), Day 28 and Day 42. Experiments were repeated 3 times per organism.

Results

At the end of the 3-hour temperature excursion, the surface temperature of units were $18.7 \pm 0.8^{\circ}\text{C}$. Bacterial results are expressed as $(\text{CFU}/\text{mL}_{\text{TEST}})/(\text{CFU}/\text{mL}_{\text{CONTROL}})$ in the table below. No significant differences were observed between the growth of test and control units during storage for *P. fluorescens*, *E. coli*, *S. aureus* *E cloacae* or *S caprae*. On Day 14 post excursion, the bacterial count in test units containing *Y. enterocolitica* declined approximately 4-fold relative to control units; no differences were observed in other timepoints. For *S. marcescens*, significantly greater levels of bacteria (3.2- and 5.4-fold) were observed in test units relative to those of controls on Days 28 and 42, respectively. Bacteria counts of *S. liquefaciens* that were subjected to the temperature excursion were 4.4-fold greater than those post-excursion of control units on Day 14, but this difference did not attain statistical significance.

Organism	log(CFU/mL) _{TEST} /log (CFU/mL) _{CONTROL}				
	Day 7	Day 14 pre	Day 14 post	Day 28	Day 42
<i>P fluorescens</i>	1.0	0.79 ± 0.40	0.69 ± 0.13	1.10 ± 0.19	0.86 ± 0.14
<i>Y enterocolitica</i>	1.0	0.79 ± 0.11	0.025 ± 0.033	0.83 ± 0.01	0.76 ± 0.11
<i>S marcescens</i>	1.0	1.27 ± 0.29	1.03 ± 0.43	3.19 ± 0.15	5.37 ± 0.43
<i>S liquefaciens</i>	1.0	1.42 ± 0.15	4.38 ± 2.58	1.32 ± 0.29	1.19 ± 0.12
<i>E coli</i>	1.0	0.94 ± 0.46	1.43 ± 0.43	No growth	No growth
<i>S aureus</i>	1.0	1.31 ± 0.60	1.00 ± 0.17	0.51 ± 0.11	1.76 ± 0.89
<i>E cloacae</i>	1.0	0.99 ± 0.25	1.29 ± 0.175	No growth	No growth
<i>S caprae</i>	1.0	0.33 ± 0.57	0.39 ± 0.35	No growth	No growth

Conclusions

A 3-hour room temperature excursion in RBCs did not affect bacterial counts in *P. fluorescens*, *E. coli*, *S. aureus*, *E cloacae*, and *S caprae*, temporarily decreased the levels of *Y. enterocolitica* immediately following temperature excursion but enhanced the growth of *S. marcescens* during later storage relative to continuous 1-6° storage.

Results/Findings

At the end of the 3-hour temperature excursion, the surface temperature of units were $18.7 \pm 0.8^\circ\text{C}$. Bacterial results are expressed as (CFU/mLTEST)/(CFU/mLCONTROL) in the table below. No significant differences were observed between the growth of test and control units during storage for *P. fluorescens*, *E. coli* and *S. aureus*. On Day 14 post excursion, the bacterial count in test units containing *Y. enterocolitica* declined approximately 4-fold relative to control units; no differences were observed in other timepoints. For *S. marcescens*, significantly greater levels of bacteria (3.2- and 5.4-fold) were observed in test units relative to those of controls on Days 28 and 42, respectively. Bacteria counts of *S. liquefaciens* that were subjected to the temperature excursion were 4.4-fold greater than those post-excursion of control units on Day 14, but this difference did not attain statistical significance.

Conclusion

A 3-hour room temperature excursion in RBCs did not affect bacterial counts in *P. fluorescens*, *E. coli* and *S. aureus*, temporarily decreased the levels of *Y. enterocolitica* immediately following temperature excursion but enhanced the growth of *S. marcescens* during later storage.

Authors

Stephen Jeffrey Wagner, PhD, Cheryl Hapip, Lenora A. Abel

American Red Cross Holland Lab

©2018 The American National RedCross Holland Lab, Rockville, Maryland, USA. ALL RIGHTS RESERVED.