Red Blood Cell Unit Exposure To Uncontrolled Temperatures For Over 30 minutes: Effects on Quality and Bacterial Growth

Dr. Sandra Ramirez-Arcos
(sandra.ramirez@blood.ca)

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Nothing to disclose
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• Project management team members:
  – Dr. Eiad Kahwash, Natasha McLaughlin, Dr. Yulia Lin, Dr. Jason Acker, Dr. Alan Tinmouth
• netCAD Ottawa (S. Ramirez lab): Bacterial studies
• netCAD Edmonton: Quality studies
• netCAD Vancouver: Production of RBC units
• Statistical analyst: Dr. Qi-Long Yi
• QMP: Craig Jenkins
Outline

• Objectives of this talk
• Background
• Development study
  – Phase I
  – Phase II
Objectives

• State the current International Standards for RBC storage

• Learn the effects of RBC exposure to uncontrolled temperatures on RBC quality and safety

• Understand what Canadian Blood Services is doing to test the validity of the standards related to the 30-minute rule
What is the 30-minute rule?

Origin

- Pick and Fabijanic (Transfusion 1971;11:213):
  - Whole blood units warmed and cooled intermittently have decreased quality when core temperature reached 10 °C
  - 10°C was reached within 45-60 min; therefore the “30-minute rule” was established
Canadian Standard Association Standard 10.10.5: RBC products can be re-issued if “controlled” temperature has been maintained or products have not been unrefrigerated for more than 30 min.

CSTM standard 5.7.7.1: “blood components may be returned to the transfusion service inventory if the temperature ….. is within … 1-10 degree Celsius for RBC units…. and the blood component has not been out of the controlled environment for longer than 30 minutes from issue”

British Council for Standardisation in Haematology (BCSH) guidelines: RBC products should be discarded if left unrefrigerated for more than 30 min because of the risk of bacterial growth.

Australian and New Zealand Society for Blood Transfusion, guidelines 5.5.1 and 5.5.2: RBC units left out of “controlled” temperature for more than 30 min should be transfused or returned to blood bank and marked as “unsuitable for use”

AABB Standard 5.24 (27th edition): RBC products can be re-issued if “appropriate” temperature has been maintained.
## Preliminary data - Survey Ontario and Quebec blood banks (Dr. Y. Lin)

<table>
<thead>
<tr>
<th>Province</th>
<th>Number hospitals surveyed</th>
<th>% responses</th>
<th>Units discarded in 2009</th>
<th># hospitals</th>
<th># units</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ontario</td>
<td>151</td>
<td>73</td>
<td></td>
<td>33</td>
<td>457</td>
<td>$159,493</td>
</tr>
<tr>
<td>Quebec</td>
<td>89</td>
<td>64</td>
<td></td>
<td>29</td>
<td>385</td>
<td>$134,365</td>
</tr>
</tbody>
</table>

Variable Interpretations of the CSA Standards

- All RBC units that are returned to the blood bank more than 30 minutes after issue are discarded regardless of the temperature
- All RBC units that are returned to the blood bank at a temperature greater than 10 degree Celsius are discarded regardless of the time spent outside of the blood bank
- All RBC units that are returned to the blood bank more than 30 minutes after issued OR at a temperature greater than 10 degree Celsius are discarded
- All units that are returned to the blood bank are discarded regardless of temperature or time spent outside of the blood bank

Different Methods Used to Take RBC Temperature

- 31%: We do not measure the temperature of RBC units
- 29%: Tempcheck
- 19%: Infrared thermometer
- 9%: Temperature sensitive labels
- 3%: Calibrated thermometer
- 9%: Other

Standards related to the 30-minute rule

- Canadian Standard Association Standard 10.10.5: *RBC products can be re-issued if “controlled” temperature has been maintained or products have not been unrefrigerated for more than 30 min*

- CSTM standard 5.7.7.1: “blood components may be returned to the transfusion service inventory if the temperature ..... is within ....1-10 degree Celsius for RBC units.... and the blood component has not been out of the controlled environment for longer than 30 minutes from issue”

- British Council for Standardisation in Haematology (BCSH) guidelines: *RBC products should be discarded if left unrefrigerated for more than 30 min because of the risk of bacterial growth*

- Australian and New Zealand Society for Blood Transfusion, guidelines 5.5.1 and 5.5.2: *RBC units left out of “controlled” temperature for more than 30 min should be transfused or returned to blood bank and marked as “unsuitable for use”*

- AABB Standard 5.24 (27th edition): *RBC products can be re-issued if “appropriate” temperature has been maintained*

Why 30 minutes? *Potential change in RBC quality and safety*
Quality risk

- Core T > 10°C are reached in less than 30 minutes of RBC units exposure to uncontrolled temperatures
- RBC quality is not significantly affected by single or multiple exposures to uncontrolled T
  - Thibault et al. AABB Meeting, Abstract SP68. Transfusion. 2012;52(Suppl.):80A

Evaluating the 4-hour and 30-minute rules: effects of room temperature exposure on red blood cell quality and bacterial growth

Sandra Ramirez-Arcos, Cherie Mastronardi, Heather Perkins, Yuntong Kou, Tracey Turner, Emily Mastronardi, Adele Hansen, Qi-Long Yi, Natasha McLaughlin, Eiad Kahwash, Yulia Lin, and Jason Acker
Experimental design

Two matched, pooled, split units: CONTROL and TEST

Day 6
- Paired with dummy units for T monitoring
- RBC quality testing
- Return to storage at 1-6°C

Days 9, 13, 14, 15, 16
On each day:
- RT exposure of test unit for 30 minutes
- Return to storage at 1-6°C

Day 20
- Remove from storage for sterility testing and final testing of RBC quality
- Download T data
Results

RBC core temperature ranged from 7.3 to 11.6°C during 30 min RT exposures.

![Canadian Blood Services: it's in you to give]
Safety risk

- Prevalence of bacterially-contaminated RBCs can be as high as ~1/30,000 with fatality rates of up to ~1/100,000 (Chen et al. Transfusion 2008;48:1550)

- CBS:
  - September 2010, confirmed report of a severe transfusion reaction due to RBCs contaminated with *Klebsiella pneumoniae*
  - December 2011, suspected transfusion reaction due to RBCs contaminated with *Klebsiella pneumoniae*
  - QC data Nov 2009 – September 2012:
    - 24,974 RBC units tested (1% units produced)
    - 6 contaminated (1/4,162 ~ 0.02%):
      » *Propionibacterium acnes* (5)
      » *Bacillus licheniformis* (1)
Safety risk

Outstanding question: What is the effect on bacterial growth in contaminated RBC units after multiple exposures to uncontrolled $T$?

  - Risk of bacterial growth in contaminated units was not real since bacterial growth did not increase after 2h RT exposure

- Thibault et al (AABB Meeting, Abstract S86-040A. Transfusion. 2012;52(Suppl.):47A)
  - Multiple 30-minute or 60-minute RT exposures did significantly influence bacterial growth in spiked RBC units

Our studies
Objective

To evaluate the effect of multiple 30-min exposures of RBC units to RT on bacterial growth
Two matched, pooled, split units: CONTROL and TEST

- Paired with dummy units for T monitoring
- Sterility testing
- Spiking of test unit with *Serratia marcescens* (100 CFU/ml)
- Return to storage at 1-6\(^\circ\)C

On each day:
- RT exposure of test units for 30 minutes
- Return to storage at 1-6\(^\circ\)C

- Remove from storage for final determination of bacterial concentration

Day 6

Day 20

Day 6

Days 9, 13, 14, 15, 16

RT = 20 ± 0.4 \(^\circ\)C

Exposed Control

Results - Growth of *S. marcescens* upon Multiple (5) 30-hour RT exposure

1.00E+00
1.00E+01
1.00E+02
1.00E+03
1.00E+04
1.00E+05

Bacterial concentration (CFU/ml)

Initial Final

$p < 0.05$

Summary – Phase I

Five 30-min exposures to RT induces significant bacterial growth by a psychrophilic strain

What is the clinical significance of these results?
Phase II
Objective

To determine if multiple 30-min and 60-min exposures of RBC units to RT would promote bacterial growth
Experimental design
Phase II

N = 2

- Control (C) unit will remain in storage
- Test (T) unit was exposed 6 times to RT, each time for 30 min (total = 3 hours) during 42 d of storage

- Control (C) unit will remain in storage
- Test (T) unit was exposed 3 times to RT, each time for 60 min (total = 3 hours) during 42 d of storage

~ 24 hours after each exposure, both control and test units of each group will be sampled to determine bacterial concentration

At 42 days of storage, all units will be sampled to determine bacterial concentration

* Serratia marcescens, Yersinia enterocolitica, Staphylococcus epidermidis and Escherichia coli

Sterility testing and units spiking to target 1 CFU/ml*
Results – *E. coli, S. epidermidis*

- No growth observed
  - Self-sterilization
Results – *Y. enterocolitica*

Comparison of overall growth

<table>
<thead>
<tr>
<th>Comparison of overall growth</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test 30 vs Control 30</td>
<td>0.61</td>
</tr>
<tr>
<td>Test 60 vs Control 60</td>
<td>0.43</td>
</tr>
<tr>
<td>Test 30 vs Test 60</td>
<td>0.85</td>
</tr>
<tr>
<td>Control 30 vs Control 60</td>
<td>0.24</td>
</tr>
<tr>
<td>All</td>
<td>0.53</td>
</tr>
</tbody>
</table>

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![Graph showing bacterial concentration over time with arrows indicating 30 min and 1 h exposure.]

= 30 min exposure

= 1 h exposure
Comparison overall growth

<table>
<thead>
<tr>
<th>Comparison</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test 30 vs Control 30</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Test 60 vs Control 60</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Test 30 vs Test 60</td>
<td>0.78</td>
</tr>
<tr>
<td>Control 30 vs Control 60</td>
<td>0.32</td>
</tr>
<tr>
<td>All</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Results – *S. marcescens*

- = 30 min exposure
- = 1 h exposure
Clinical significance of these results?
Endotoxin

Lipopolysaccharide [LPS; 'Endotoxin']

Polysaccharide Surface Layer

Outer Membrane

Periplasm

Cytoplasmic Membrane

Cytoplasm

Capsular Polysaccharide (Some Species or Strains)

Enterobacterial Common Antigen

'Lipoglycans'

Phospholipid

Lipoprotein

Peptidoglycan

Phospholipid

Protein


http://medical-dictionary.thefreedictionary.com/endotoxin
Endotoxin

- Interleukin-1 and interleukin-2
- Histamine
- Tumor necrosis factor
- Activation of coagulation system
- Myocardial depressant factor
- Prostaglandin, thromboxane, leukotriene, and prostacyclin release
- Anaphylatoxins C5a and C3a
- Beta-endorphins
- Platelet-activating factor
- Oxygen-derived free radicals
- Bradykinin
Results – *Y. enterocolitica*

Endotoxin production *Y. enterocolitica* in RBC units

**Results**

- *Y. enterocolitica*
Results – *S. marcescens*

Endotoxin production *S. marcescens* in RBC units

- Median Endotoxin units (EU)/ml vs. Days post collection
- Lines denote different time points: 4C, 30min, 60min

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*Canadian Blood Services*  
it's in you to give
### Bacterial concentration vs. Endotoxin levels

$>10^5$ CFU/ml (11,373 – 182,700 EU) related to transfusion reaction in platelets (Jacobs et al, CID 2008;46:1214)

<table>
<thead>
<tr>
<th>CFU/ml</th>
<th></th>
<th>Endotoxin (EU) in RBC units (~250 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$S.\ marcescens$</td>
</tr>
<tr>
<td></td>
<td>Per ml</td>
<td>Total</td>
</tr>
<tr>
<td>$1.00E+01$</td>
<td>≤0.03</td>
<td>0.27</td>
</tr>
<tr>
<td>$1.00E+02$</td>
<td>≤0.03</td>
<td>6.75</td>
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<tr>
<td>$1.00E+03$</td>
<td>50</td>
<td>13,556</td>
</tr>
<tr>
<td>$1.00E+04$</td>
<td>75</td>
<td>19,235</td>
</tr>
<tr>
<td>$1.00E+05$</td>
<td>1000</td>
<td>358,847</td>
</tr>
<tr>
<td>$1.00E+06$</td>
<td>50000</td>
<td>13,384,231</td>
</tr>
<tr>
<td>$1.00E+07$</td>
<td>&gt;60000</td>
<td></td>
</tr>
<tr>
<td>$1.00E+08$</td>
<td>&gt;60000</td>
<td></td>
</tr>
</tbody>
</table>
Summary - Phase II

- Mesophilic bacteria (E. coli and S. epidermidis) do not proliferate under RBC storage conditions despite RT exposure.

- Psychrophilic bacteria:
  - Growth and endotoxin production of Y. enterocolitica is not affected by RT exposure.
    - Therefore not added risk by RBC unit warming.
  - Overall growth of S. marcescens is increased by RT exposures but
    - There is no difference in growth or endotoxin production between units exposed for 30 minutes or for 60 minutes.
    - Therefore not added risk by extending RBC exposure to RT.

- Similar results by HQ
  - AABB Meeting, Abstract S86-040A. Transfusion. 2012;52(Suppl.):47A

An extension of the 30-minute rule to 60 minutes is feasible in Canada.
Thank you for your attention

Questions?