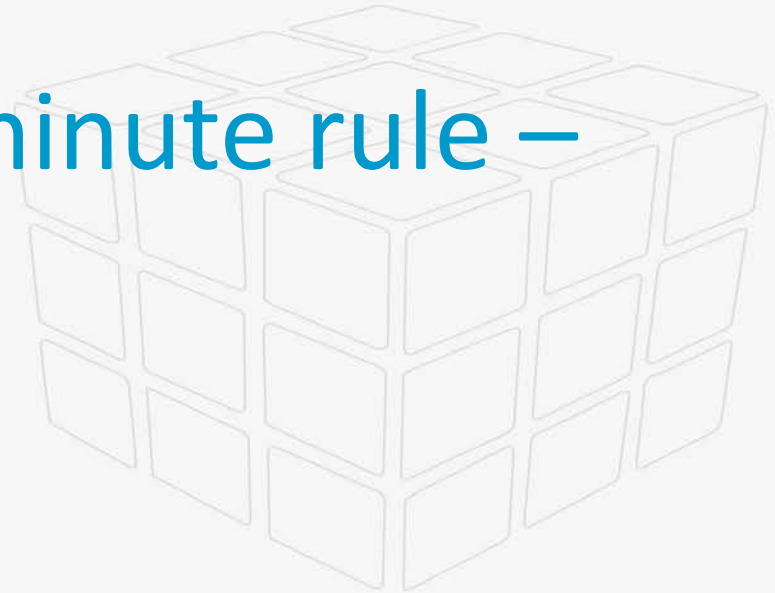




Extending the 30 minute rule – It's about time...



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Outline

- Background
- Study Design
- Results
- Repeat Work
- Conclusions



The '30 minute rule'

*Red cells removed from CTS for transfusion
must be returned to storage within 30 minutes
if no longer required*

Why 30 minutes?

- Current UK guidelines stipulate storage of red cells at 2 – 6° C
- Reduces red cell metabolism & helps to maintain quality throughout storage
- Risk of bacterial proliferation increases with time when units are stored at ambient temperatures

Evidence behind 30 minutes

- The 1971 publication indicated 30 minutes as being appropriate:
 - **SURFACE** 15 – 30 minutes to reach 10° C
 - **CORE** 45 – 60 minutes to reach 10° C
 - when stored at room temperature
 - whether handled or not
- 30 minutes was accepted as a compromise to ensure core does not reach 10° C



**KEEP
CALM
WE'VE GOT
30
MINUTES**

Problems with the '30 minute rule'

- Not always possible to commence transfusion within the stipulated timeframe
- Extremely wasteful – disposal of large numbers of red cells
- Huge cost to the NHS





**BUT
WOULD
60 MINUTES
BE BETTER?**

Purpose of the NBL Study

- To validate the 30 minute rule
- To explore the possibility of extending the rule
- Investigation of multiple out of CTS exposures

AND

- To compliment work performed by NHSBT Components Development Laboratory (CDL)

Thomas, S. *et al.*, (2013) *Transfusion* (53), 1169-1177

NBL Pilot Study (2012)

- Trial run using 2 test organisms
- 30° C exposures for 30, 60, 120 minutes
- Indicated the following for a full study:
 - Inclusion of paediatric units
 - Less temperature deviations
 - Use of data loggers
 - Match time out of CTS with time returned before further exposures

NBL Full Study (2013)

- Study design was defined by CDL work:
 - Out of CTS exposure days (15, 17, 21)
 - 30° C exposure chosen as 'worst case scenario'
 - 30° C exposure times (30 / 60 minutes)
 - Frequency of exposures (1 or 3)
 - Multiple exposures on one day
 - Testing adult AND paediatric units

Test Organisms

Organism	Component	Frequency	Patient outcome
<i>Serratia liquefaciens</i>	Transmission (1997; Red cell) ATCC 35551	1	Morbidity
<i>Yersinia enterocolitica</i>	Transmission (1999; Red cell) ATCC 27729	1	Death
<i>Staphylococcus epidermidis</i>	Transmission (1999; Red cell)	1	Morbidity
<i>Bacillus cereus</i>	Transmission (2000; Pooled platelet)	1	Death
<i>Enterobacter cloacae</i>	Transmission (2007; Red cell)	1	Morbidity
<i>Pseudomonas putida</i>	Transmission (2007; Red cell)	2	Death (1) Morbidity (1)

Study Design

12 pooled



Day 1
16 RCC

4 pooled



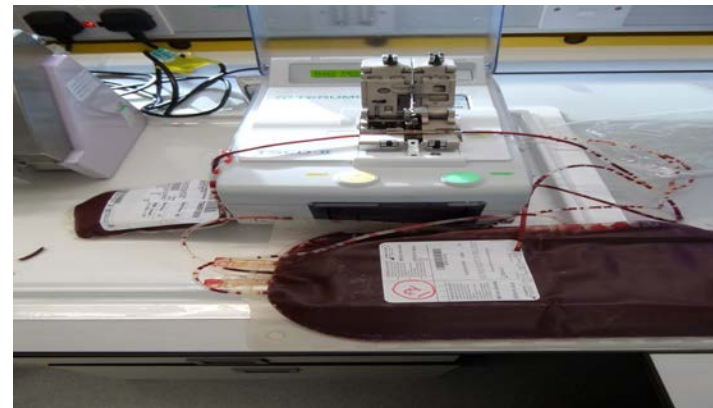
Split into:

**8 Adult
8 Paediatric
4 Negative Controls**
(2 x A, 2 x P)

**Positive
Controls**
(2 x A, 2 x P)

Adult unit size = ~250ml

Paediatric unit size = ~60ml



Study Design

- A 10^3 cfu/ml suspension of the test organism was prepared
- All 24 units were spiked at ~1:100
- Mixed well by inversion
- All units sampled for enumeration
- Units stored at 2 – 6° C or 30° C

Study Design

Unit Type		Test Day						
Adult	Paediatric	1	7	15	17	21	28	35
A	E	Spike & sample all units	Sample all units	30 x 1	30 x 1	30 x 1	Sample all units	Sample all units
B	F			30 x 3	-	-		
C	G			60 x 1	60 x 1	60 x 1		
D	H			60 x 3	-	-		
Positive	Positive			30° C	30° C	30° C		
Negative	Negative			CTS	CTS	CTS		

Study Design – Day 15

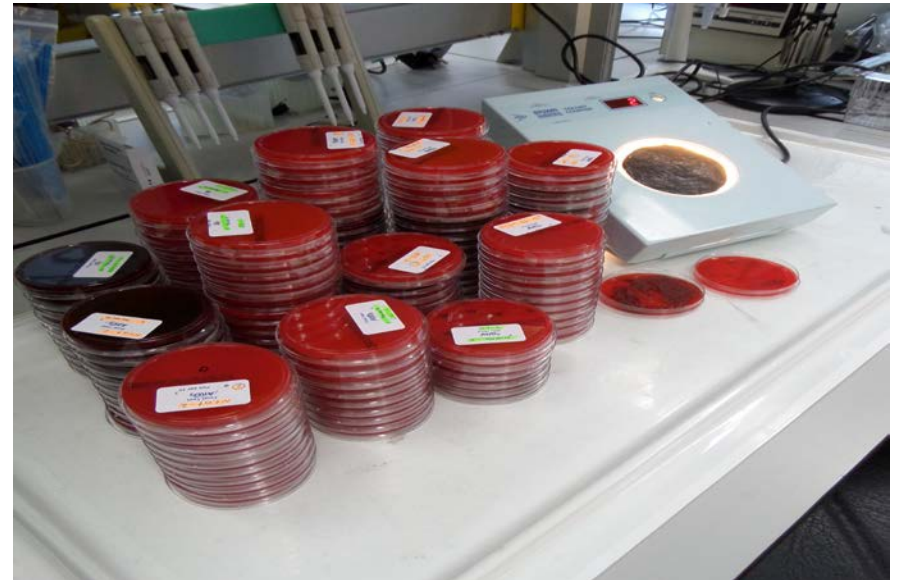
Time	A	B	C	D	E	F	G	H	NEG	POS
0830	T	T	T	T	T	T	T	T	T	T
0900	T	T			T	T				
0930	T	T	T	T	T	T	T	T	T	
1000		T				T				
1030		T	T	T		T	T	T	T	T
1100		T				T				
1130		T		T		T		T	T	
1200										
1230				T				T	T	T
1300										
1330				T				T		
1400										
1430				T				T	T	T

Study Design – Days 17 & 21

Time	A (30)	C (60)	E (30)	G (60)	NEG	POS
0830	T	T	T	T	T	T
0900	T		T			
0930	T	T	T	T	T	
1000						
1030		T		T	T	T

Study Design

- Samples were taken from all units at the defined time points
- Diluted & enumerated
- Plates incubated, then colonies counted...
- Viable counts recorded & compared with the Negative control



An increase in bacterial number of $\geq 1 \log_{10}$ in test over controls was considered significant

Results

Mesophilic Organisms

Bacillus cereus

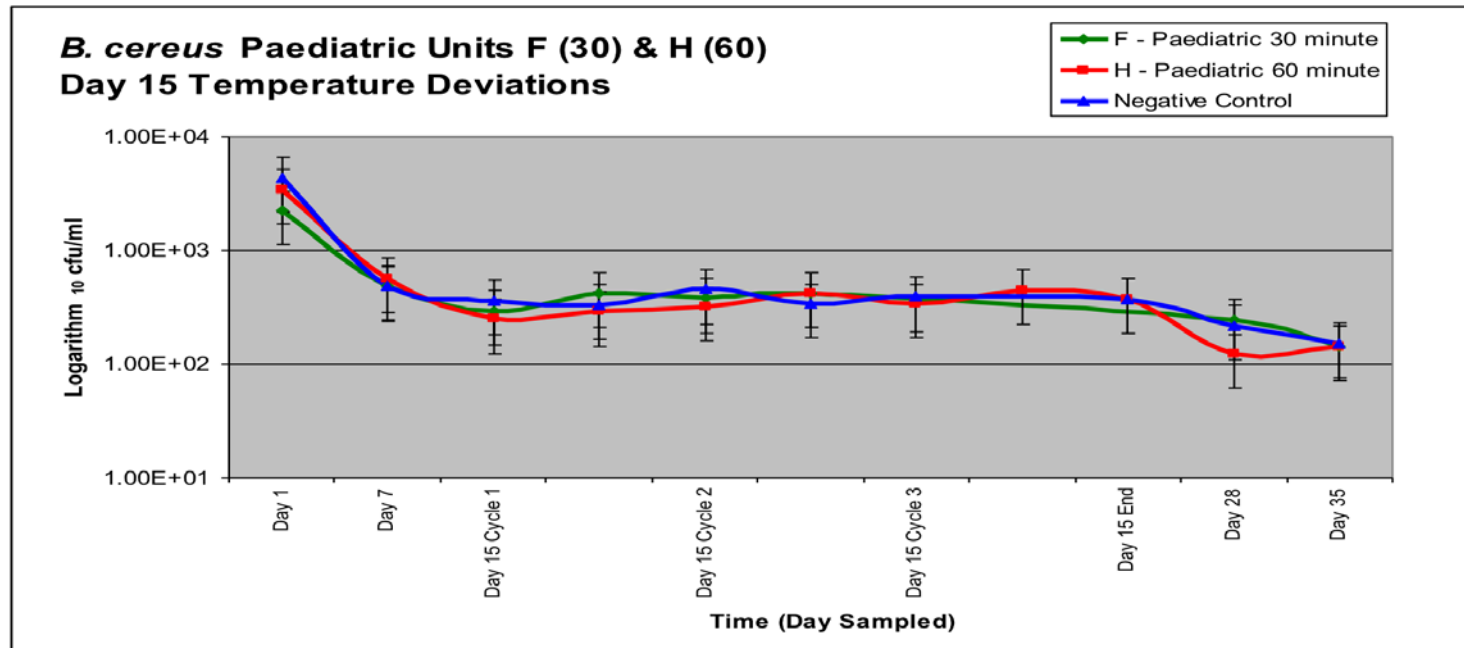
- There was little impact on bacterial counts, a gradual reduction in growth after spiking was observed

Staphylococcus epidermidis

- Some impact was noted on bacterial growth by day 35 of the experiment for adult units only ($> 1 \log_{10}$ difference from control)

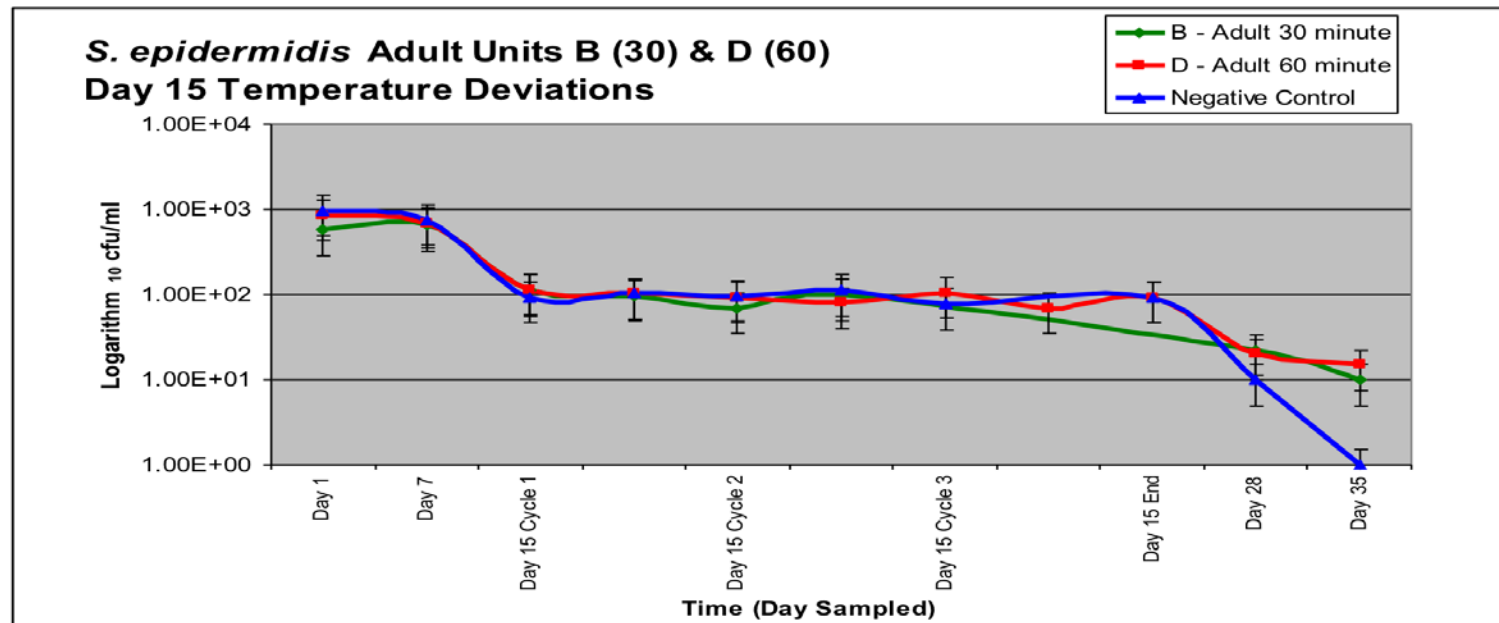
Bacillus cereus

Paediatric units exposed to 30° C for 30 or 60 minutes, three times on day 15



Staphylococcus epidermidis

Adult units exposed to 30° C for 30 or 60 minutes, three times on day 15

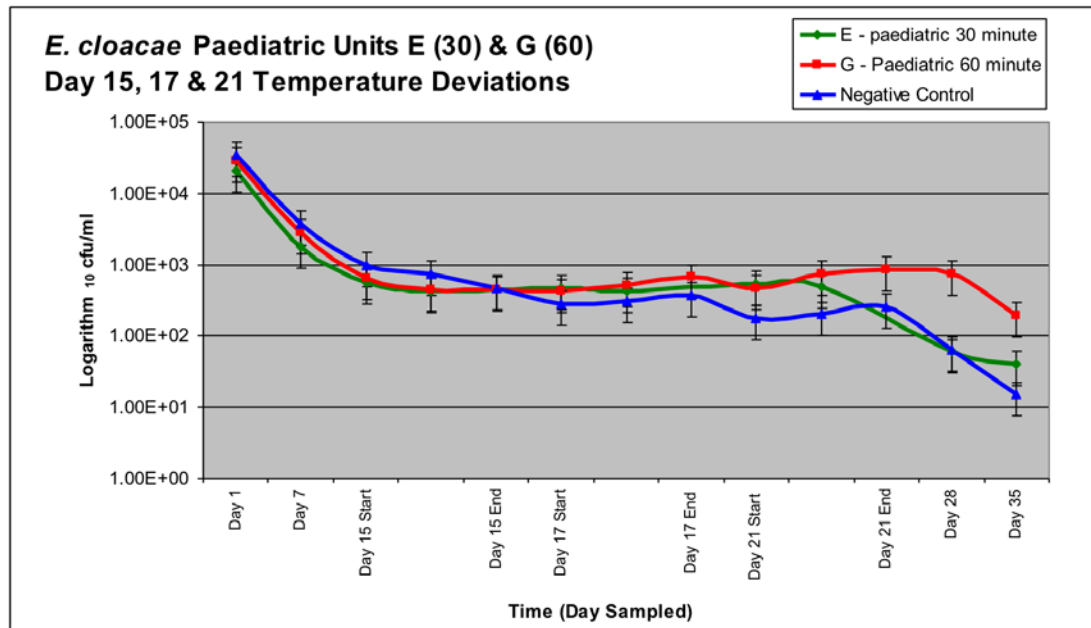


Results

Mesophilic Organisms

Enterobacter cloacae

- $> 1 \log_{10}$ difference from control noted on days 28 and 35 (paediatric units)

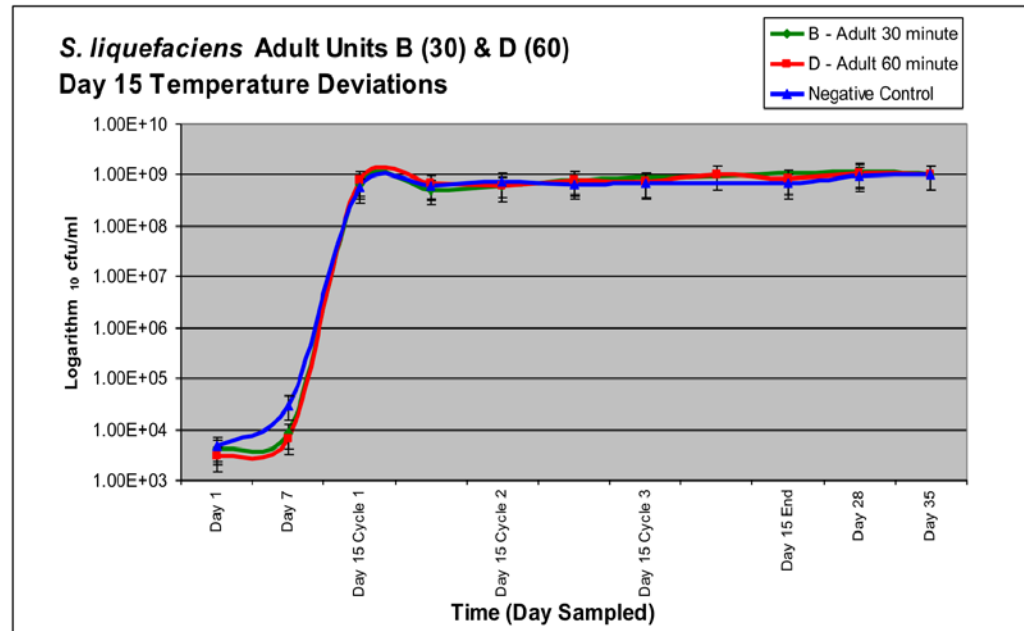


Results

Psychrophilic Organisms

Serratia liquefaciens and *Yersinia enterocolitica*

- Temperature deviations had little or no effect on the bacterial counts, the test AND control reached growth levels of $10^8 - 10^9$ cfu/ml



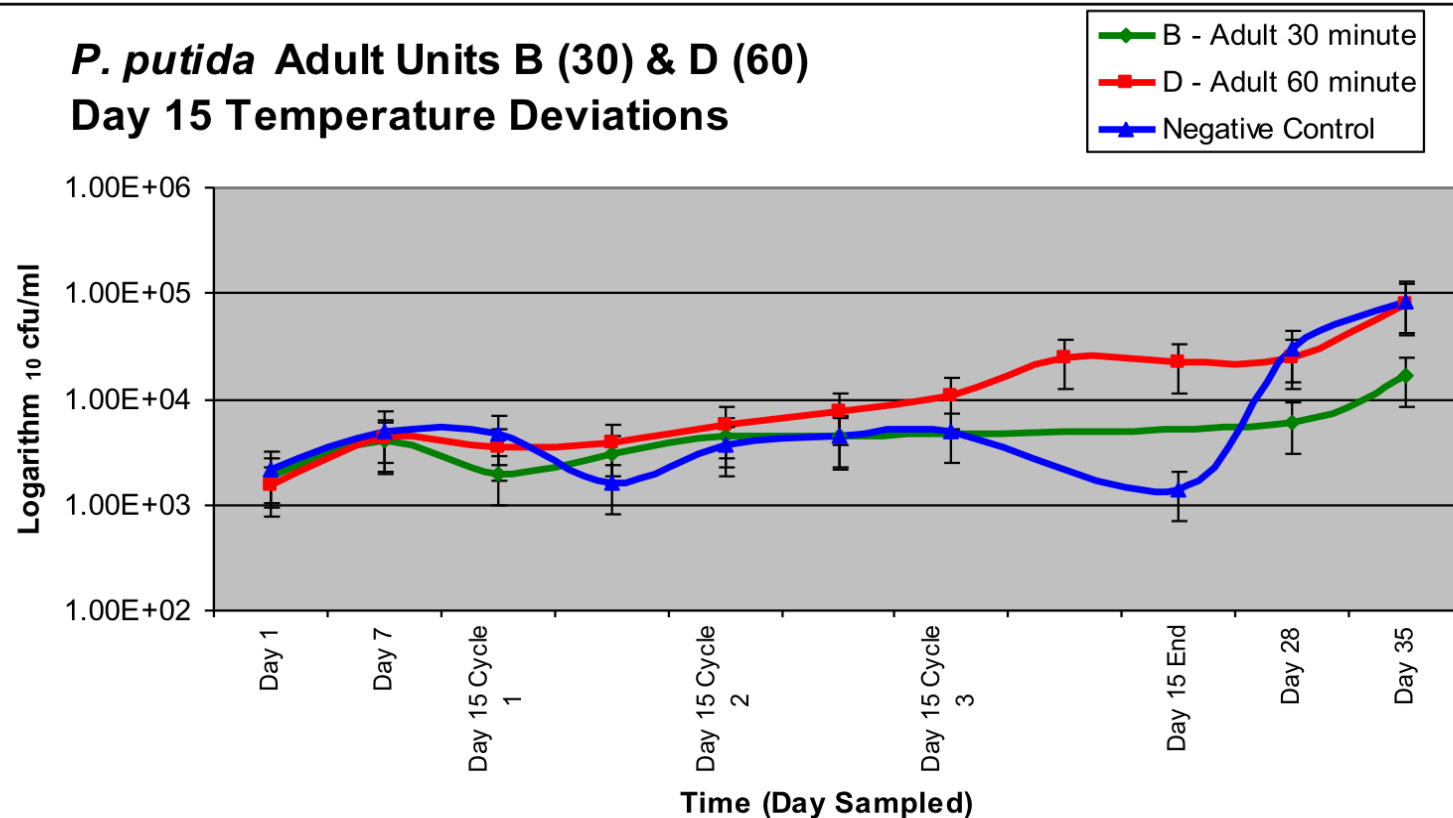
Results

Psychrophilic Organisms

Pseudomonas putida

- Considerable impact was noted during the 1st and 2nd rounds of testing
 - Multiple exposures on a single day presented a risk
 - Proliferates well at refrigeration temperatures
 - Raised some concern during previous NBL studies

***P. putida* Adult Units B (30) & D (60)** **Day 15 Temperature Deviations**



Work in 2015

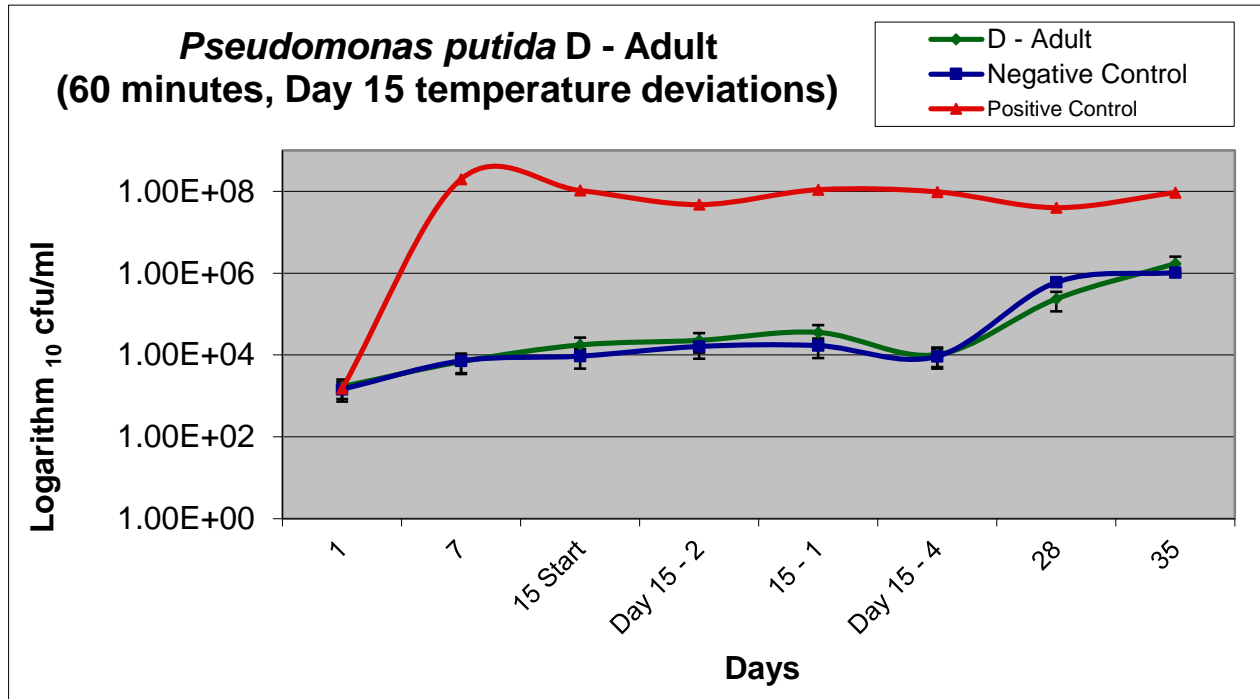
- In October 2015 we started some more work on the project.
 - Retesting:
 - *Serratia liquefaciens*
 - *Yersinia enterocolitica*
 - *Pseudomonas putida*
- Lower starting inoculum
- Testing Adult units only

Work in 2015

- *P. putida* was inoculated at $\sim 10^3$ cfu/ml as previously
- We tested only Adult packs B and D, adult negative and positive controls
- Results were consistent with negative control units. The organism was able to proliferate at refrigeration temperatures, but temperature deviations had NO significant effect upon bacterial growth

2015 Results

Psychrophilic Organisms – *P. putida*

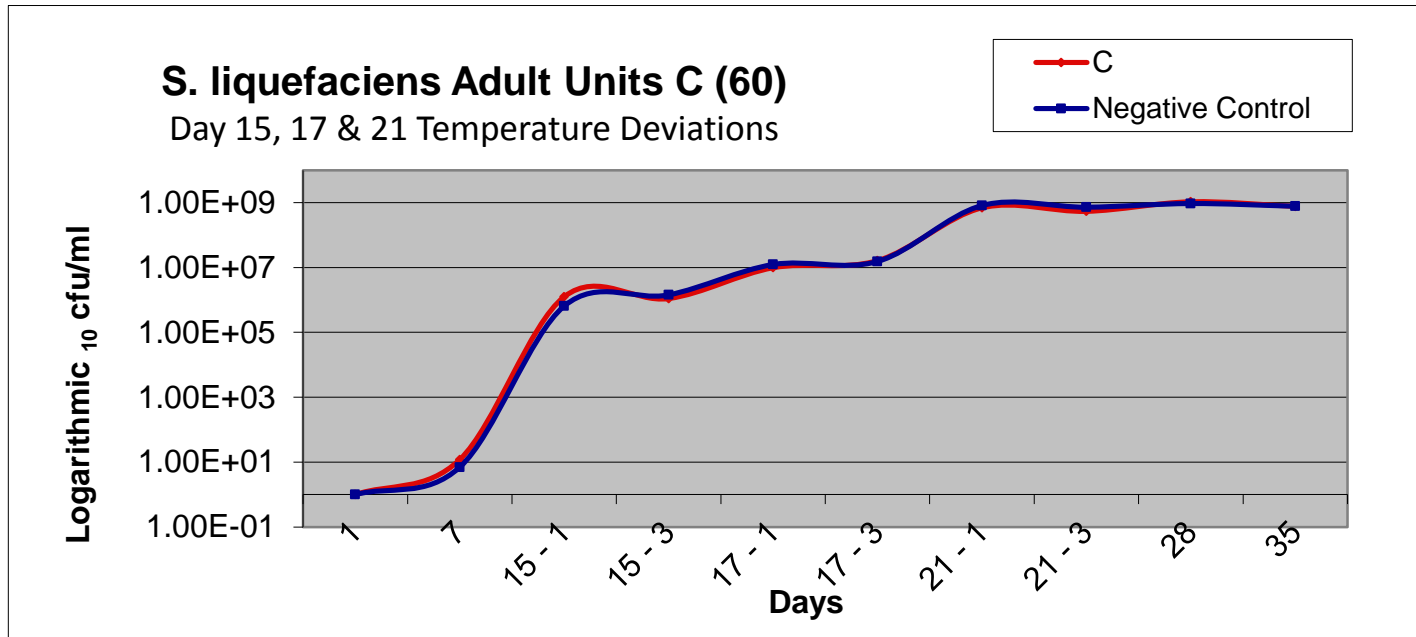


Work in 2015

- *S. liquefaciens* and *Y. enterocolitica* inoculated at ~10 cfu/ml
- To catch both organisms on Day 15 still in the exponential growth phase
- Results were consistent with negative control units
- Both organisms were able to proliferate at refrigeration temperatures, but temperature deviations had NO significant effect upon bacterial growth

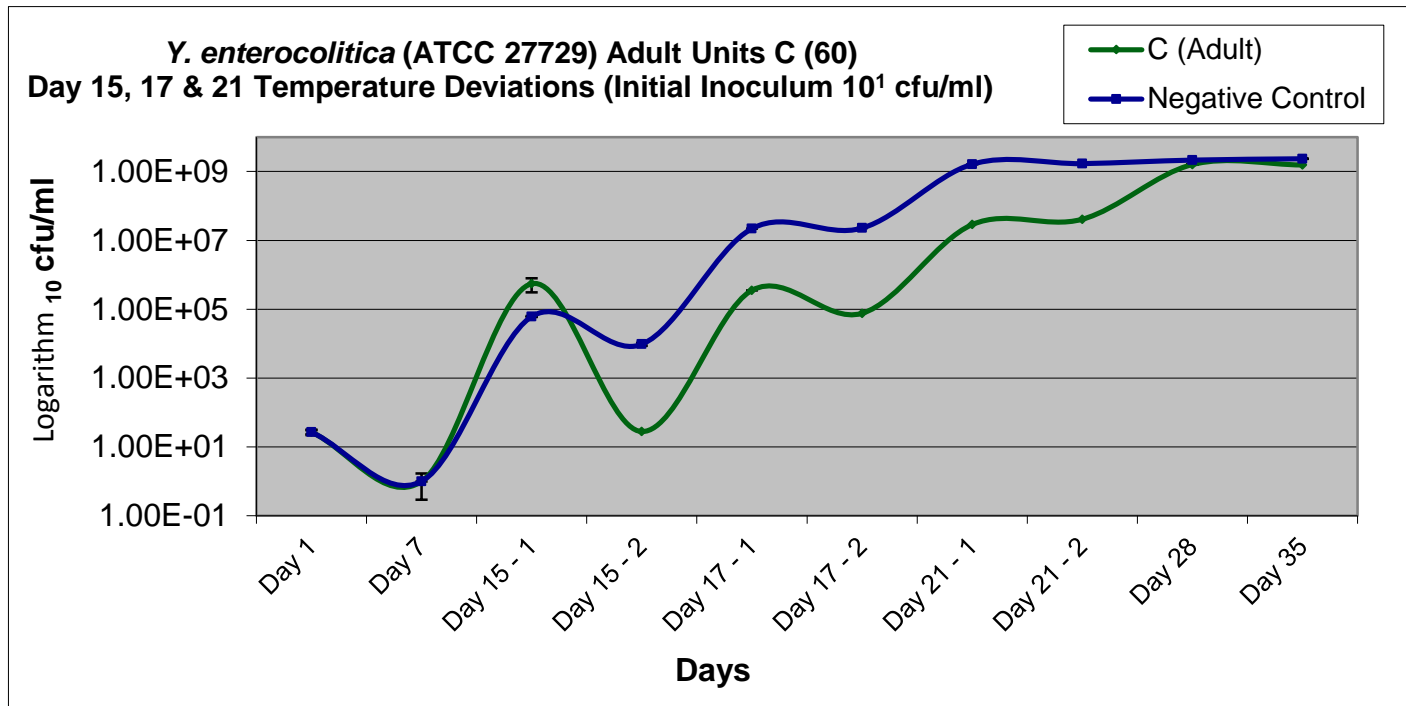
2015 Results

Psychrophilic Organisms – *S. liquefaciens*



2015 Results

Psychrophilic Organisms – *Y. enterocolitica*



Conclusions

- 30 minute rule has been validated
 - The organisms tested by NBL indicated no risk of increased bacterial number following a single 30 minute 30° C exposure
- 30 minute rule has been challenged
 - Multiple 30 and 60 minute 30° C exposures tested – with varying results
 - Indications are that extending the rule to 60 minutes would be acceptable

Conclusions

- Sufficient time must be allowed between multiple exposures for red cells to return to 2 – 6° C
 - At least an equivalent time period



Kate Aplin, NBL

Acknowledgements

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- Carl McDonald and Tyrone Pitt
- National Bacteriology Laboratory staff

A microscopic view of blood cells, showing numerous red blood cells (erythrocytes) as orange-red discs, with a few blue-stained white blood cells (leukocytes) and small green and blue granules scattered throughout.

Thank you for listening!

- **Any questions?**