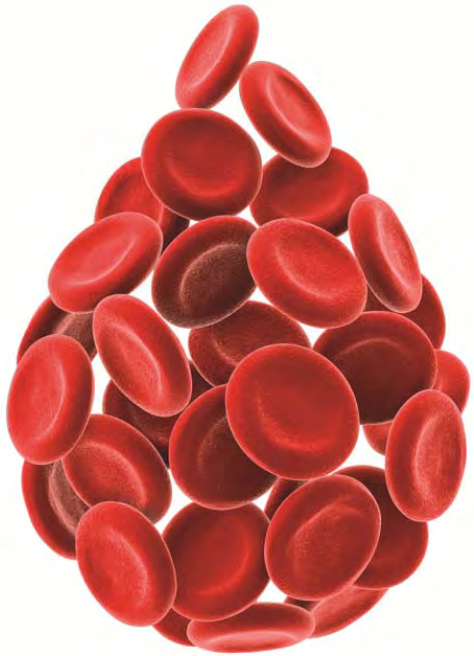


Bacterial inactivation claims in the context of sterility

A follow up on the NBL PI validation study with the INTERCEPT system

Adonis Stassinopoulos, Ph.D

V.P. Global Scientific Affairs and Research
Cerus Corporation

ISBT TTID, June 17, 2017

CERUS

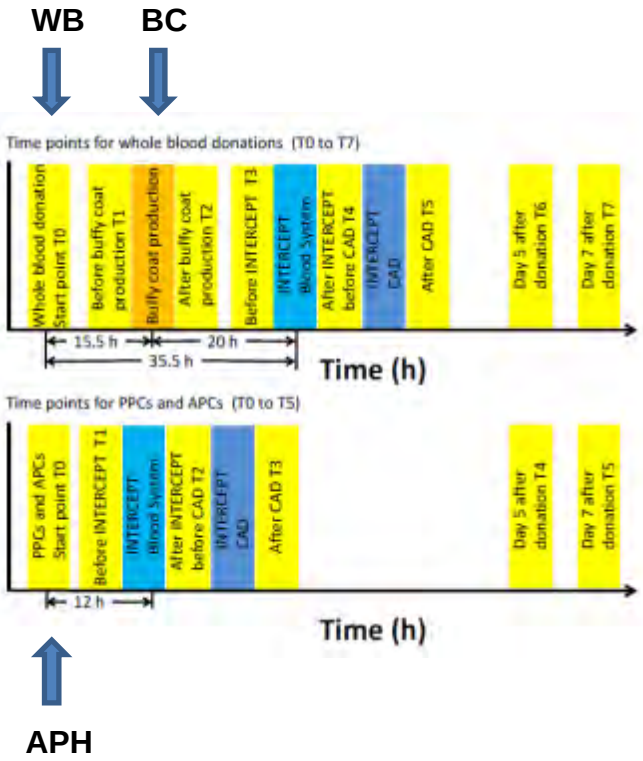
Inactivation Claims for a Broad Spectrum of Bacteria in Platelet Concentrates

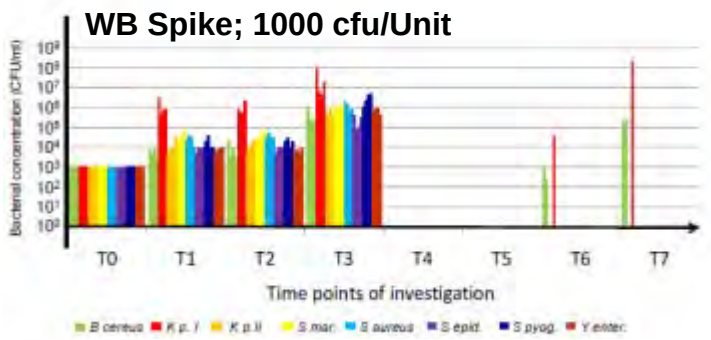
Gram-positive (Aerobes and Anaerobes)	PC/PAS	PC/PL	Gram-negative	PC/PAS	PC/PL
Staphylococcus epidermidis	> 6.6	>7.4[1]	Escherichia coli	> 6.4	≥7.3
Staphylococcus aureus	6.6	>7.6	Serratia marcescens	> 6.7	>7.1
Streptococcus pyogenes	> 6.8	>6.1	Klebsiella pneumoniae	> 5.6	≥6.7
Listeria monocytogenes	> 6.3	>6.6	Pseudomonas aeruginosa	4.5	≥6.8
Corynebacterium minutissimum	> 6.3	>6.4	Salmonella choleraesuis	> 6.2	>5.9
Bacillus cereus (vegetative)	> 6.0	≥5.6	Yersinia enterocolitica	> 5.9	>7.3
Lactobacillus sp	> 6.9	>6.1	Enterobacter cloacae	5.9	≥6.0
Bifidobacterium adolescentis	> 6.5	-	**Spirochetes**		
Propionibacterium acnes	> 6.7	>6.7	Treponema pallidum	6.8 - 7.0	-
Clostridium perfringens	> 7.0	>6.0	Borrelia burgdorferi	> 6.8	-

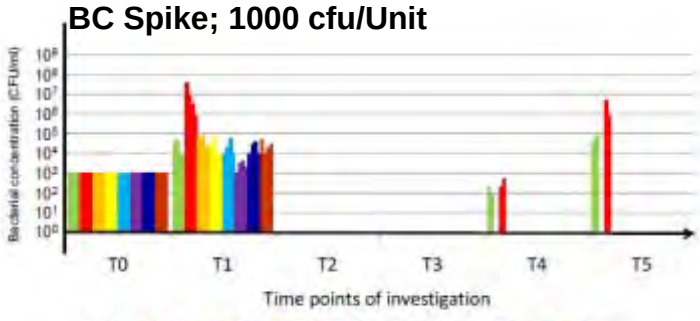
- **Robust Inactivation of Bacteria is critical to protect against them, as bacteria can grow in blood products**
- [1] In blue latest claims that have not been reviewed by TUV

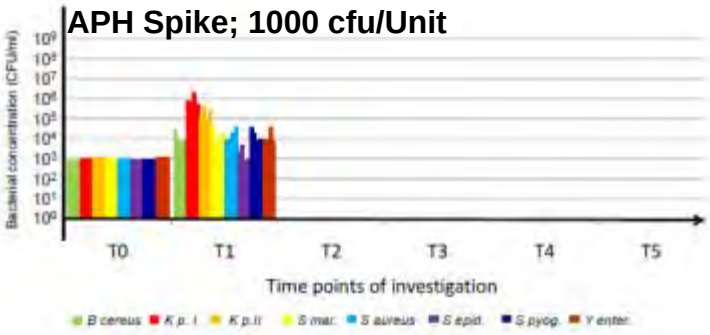
Lin et al: Transfusion 2004;44:1496-1504 and Singh et al: Transfusion 2006;46:1168-1177

Studies on bacterial inactivation with sterility endpoints- Schmidt Study



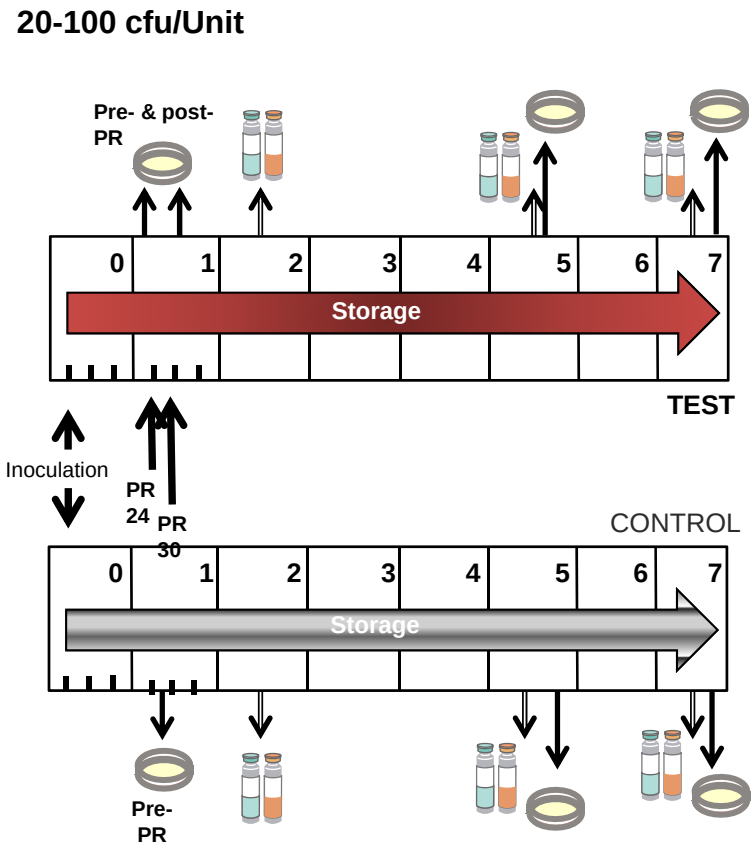




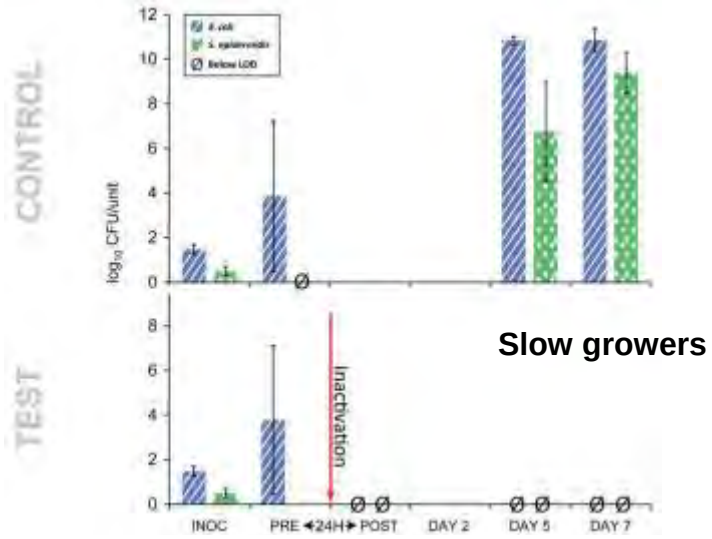


Schmidt_et.al TRANSFUSION 2015;55;2104–2112

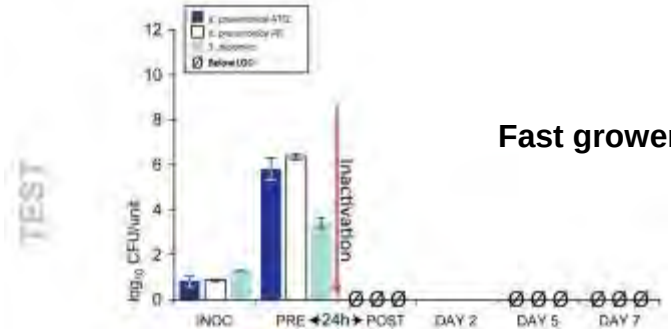
Studies on bacterial inactivation with sterility endpoints- Wagner Study



24h



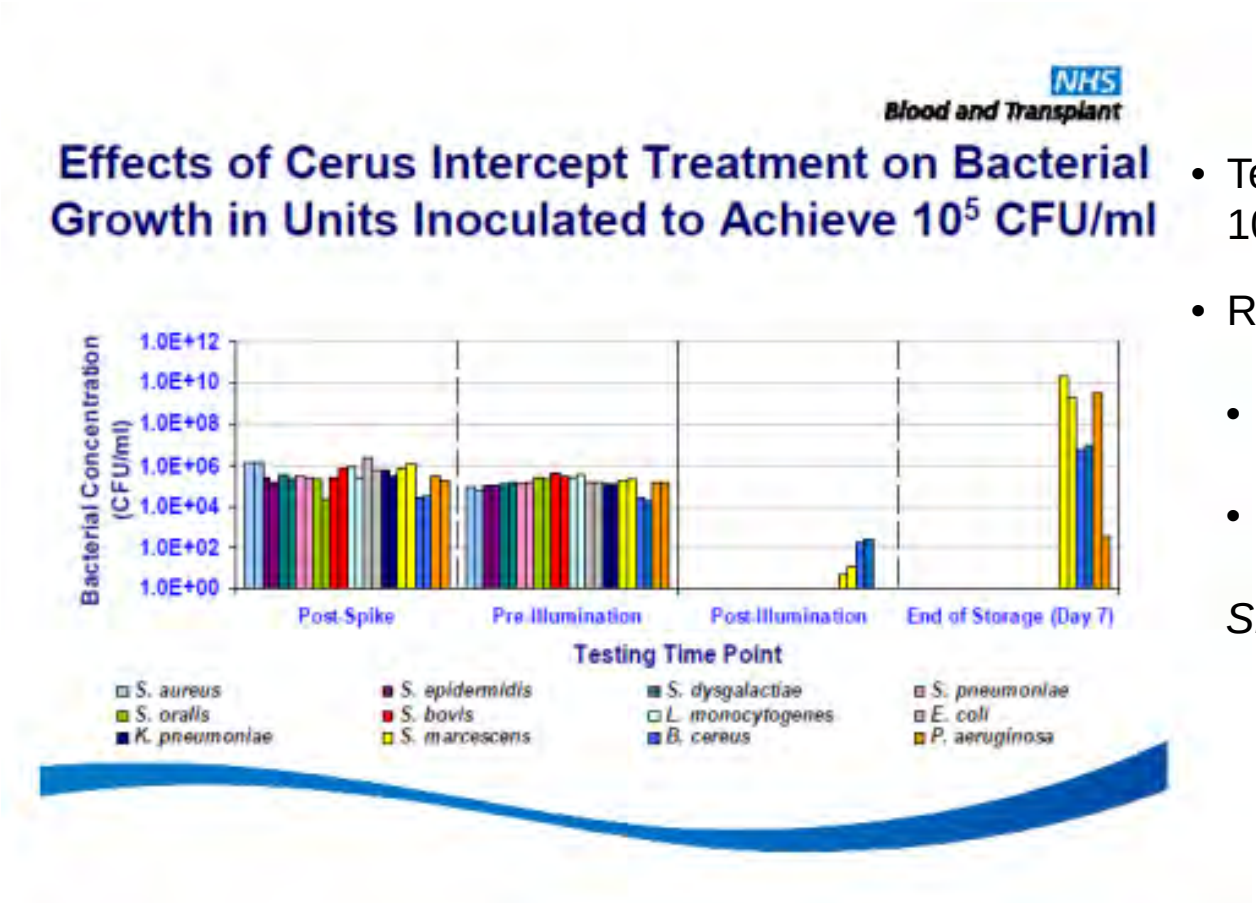
Slow growers



Fast growers

[1] Wagner et al.,Vox Sang. **2016** Oct;111(3):226-234

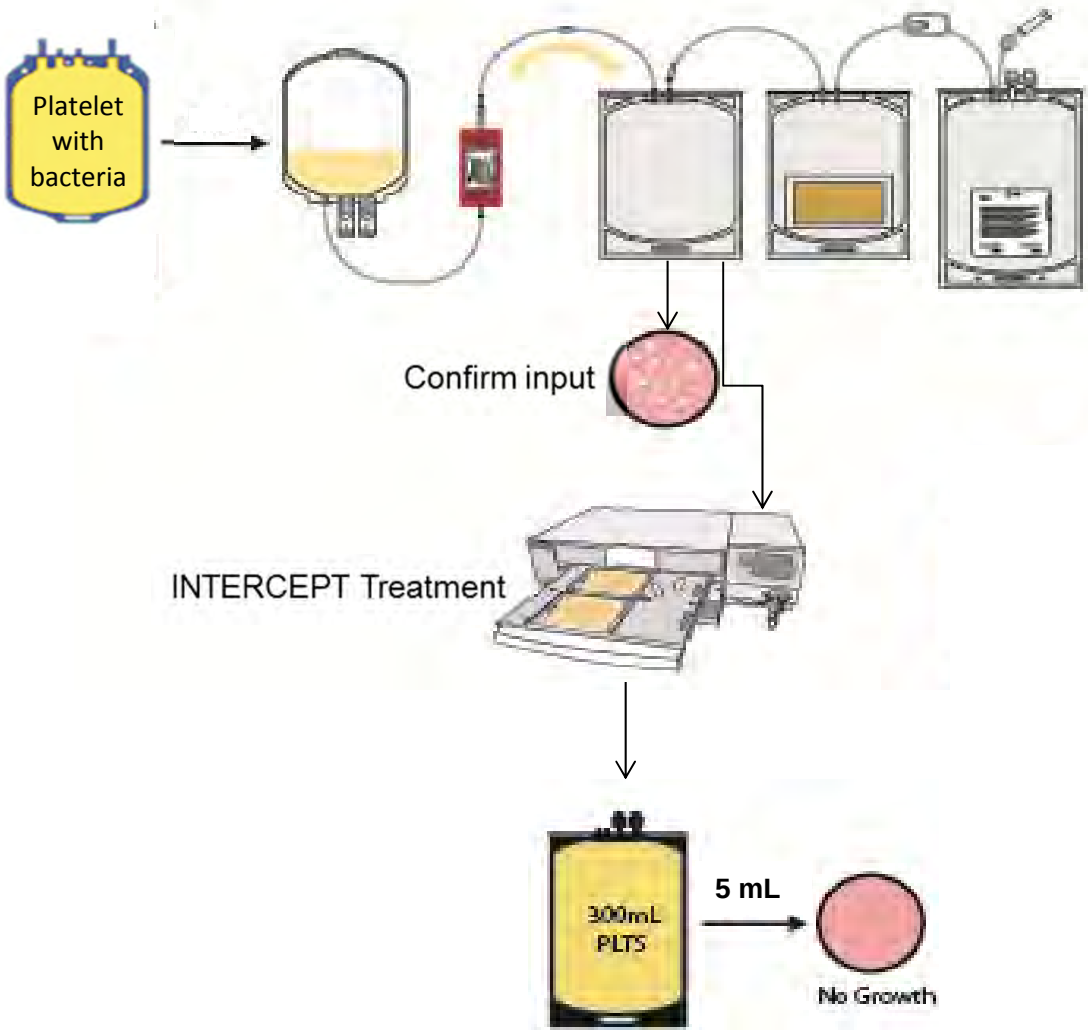
Results of the NBL evaluation for the INTERCEPT Blood System for Platelets (ISBT Dubai, 2016)



- Tested 12 bacterial strains at 10^{-1}, 10^{3} and 10^{5} cfu/mL
- Reported :
  - Good Overall Killl
  - Reduced Levels of

  S. marscecens and *P. aeruginosa*

Outline of Inactivation Study for Regulatory Submissions (Claim Studies)



- Attach bacteria contaminated platelet unit to the S-59 tube of SV Set.
- Transfer Platelets and S-59 to the illumination bag.
- Take a sample from the illumination bag for Pre-UVA bacterial titer determination and HPLC analysis.
- Transfer unit to the illuminator and expose to UVA (3J/cm^2)
- Immediately after treatment, withdraw a 5mL sample from each unit for bacterial titer determination and HPLC analysis.
- Claim based on volume tested

Measurement of Pathogen Inactivation

- **Titers are measured in log scale**

  1000 pfu/mL = 10^3 pfu/mL = 3.0 log pfu/mL

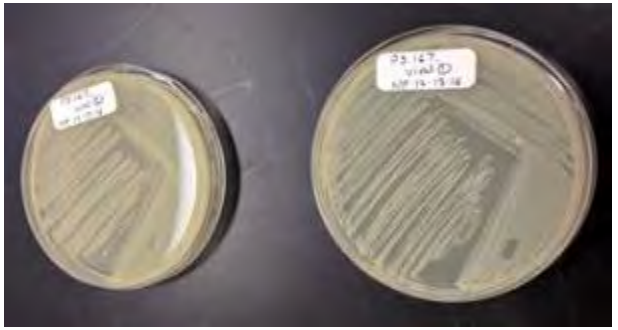
- **Inactivation is measured by log reduction, not percentages and is unit-less**
- **The volume tested is taken into account, which is important for post-inactivation limit of detection**
- **When a > is used , it means that we have exceeded the limit of detection of the system, i.e. volume tested was sterile**

Some Examples:

Pre-treatment titer (log pfu/mL)	Post-treatment titer (pfu)	Volume Tested (mL)	Post-treatment titer (log pfu/mL)	Log Inactivation
5	1	1	0	5
5	1	10	-1	6
5	1	100	-2	7
5	1	300	-2.47	7.47

The NHSBT Bacterial Strain was grown and characterized

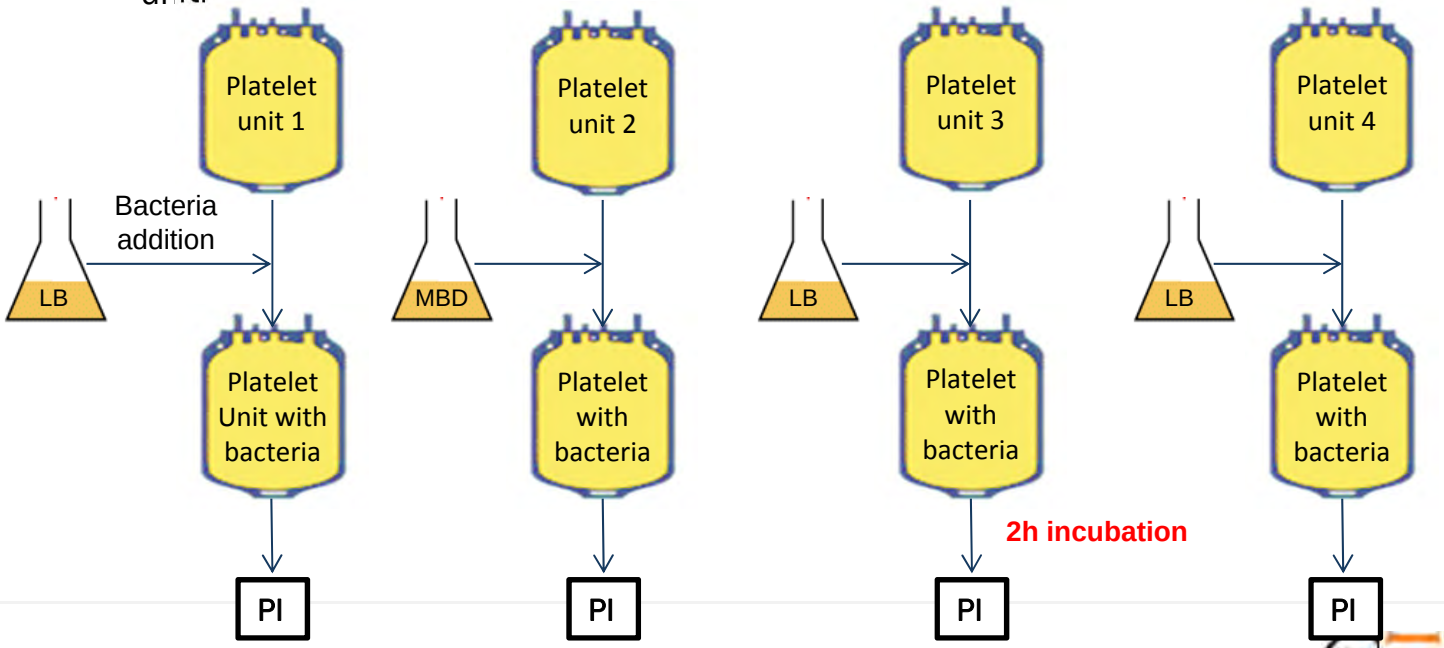
- *Bacteria samples* were received from NHSBT
- Bacterial culture from each vial was streaked on non-selective agar media (LB agar) plates.
- Culture from both the vials looked similar with homogeneous Colony morphologies, cream colored and non-pigmented
  - (LB, 37ºC)
- Samples were confirmed by 16s DNA and FAME confirmation to be *Serratia marcescens* (@ species level)



CERUS

Pathogen Inactivation studies were performed as per standard design for RA submission

- Four independent replicate experiments were performed
    - Apheresis Platelets in PAS under nominal conditions
- Bacterial cultures were prepared as per Cerus procedure
    - Single colony of bacterial culture was inoculated into either LB broth or MBD broth and the flask was incubated overnight at 37°C with sufficient aeration and agitation.
    - Stationary phase culture was added to the PC unit targeting ~6 log CFU/mL of bacteria in the unit.



Serratia marcescens Inactivation data

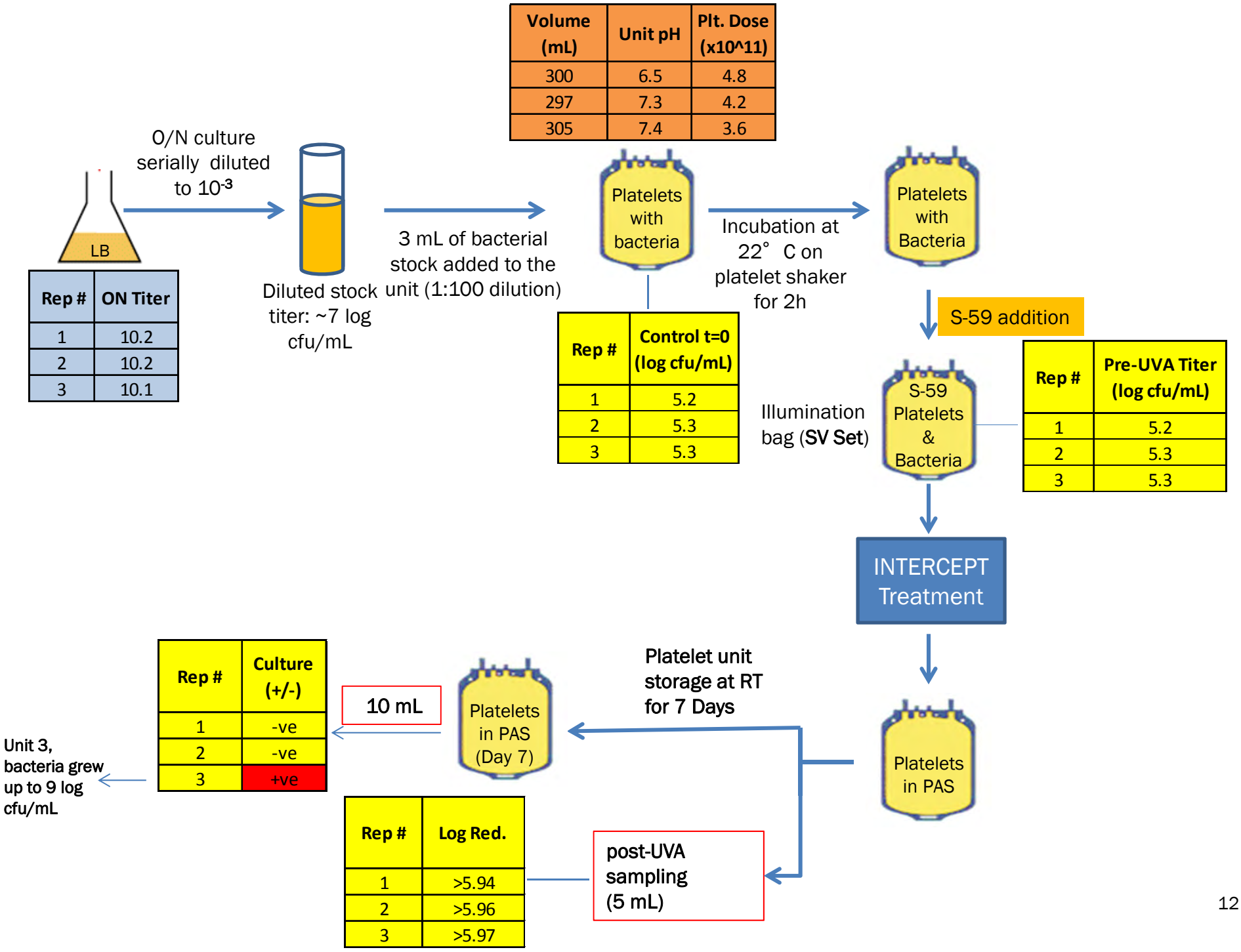
Rep #	Media used	Processing parameter (Vol. & Dose)	Input Titer Log_{10} CFU/ mL	Test Titer	Log_{10} Reduction	HPLC Pre-UVA (S-59 µM)	HPLC Post-UVA (S-59 µM)
1	LB Broth	300 mL, 2.7	**7.0**	<-0.7	**>7.7**	138	34
2	MBD	296 mL, 3.6	**7.0**	<-0.7	**>7.7**	147	38
*3	LB Broth	297 mL, 3.2	**6.8**	<-0.7	**>7.5**	142	38
4	LB Broth	294 mL, 3.2	**7.0**	<-0.7	**>7.7**	145	34

* Unit incubated for ~2 hours at 22ºC on platelet shaker prior to INTERCEPT treatment

- Inactivation to the limit of detection.
- No effect of growth medium on inactivation.
- No effect if treated immediately after bacteria inoculation versus 2 h RT pre-incubation of bacteria with platelets

Pathogen Inactivation studies were performed to emulate the NHSBT experimental design

- Preparation of Overnight culture of *S. marcescens* (NHSBT strain)
  - Overnight stock was grown in LB at 35 ^{0}C from single bacterial colony
  - The overnight culture was diluted to desired titer (~ 7log cfu/mL)
  - The bacterial stock was added to the platelet unit at 1:100 dilution to achieve 5 log cfu/mL
  - A **sample** was withdrawn from the unit to confirm the titer (T=0)
- Platelet unit incubation post inoculation
  - Bacterially inoculated platelet units were incubated at 22°C for 2 h on shaker
- Addition of S-59 to the unit
  - After incubation, the units were dosed with amotosalen
  - A **sample** was taken from the unit, for Pre-UVA titer determination (Pre-UVA Control 0J,)
- INTERCEPT Treatment
  - Platelet units were exposed to UVA (3J/cm^2).
  - A 5mL **sample** was tested Post-illumination (Post-UVA Test 3J), no viable colony observed
- Incubation of Test unit at 22°C on platelet shaker for 7 Days
  - A 10 mL of Test **sample** was tested



Rep #	ON Titer
1	10.2
2	10.2
3	10.1

Volume (mL)	Unit pH	Plt. Dose (x10^11)
300	6.5	4.8
297	7.3	4.2
305	7.4	3.6

Rep #	Control t=0 (log cfu/mL)
1	5.2
2	5.3
3	5.3

Rep #	Pre-UVA Titer (log cfu/mL)
1	5.2
2	5.3
3	5.3

Rep #	Culture (+/-)
1	-ve
2	-ve
3	+ve

Rep #	Log Red.
1	>5.94
2	>5.96
3	>5.97

Result

- All bacterially contaminated units were negative immediately post UVA, corresponding to >5.9 log inactivation
- One out of three units showed growth after 7 Day incubation
- Two out of three units were inactivated without growth after 7 days, indicating >7.7 log of inactivation

Rep #	Unit Volume (mL)	Plt. Dose (x10^11)	Control t=0	Control Pre t=0	Control Titer per Unit	Test	Log Reduction	Day 7 growth	Log Reduction per unit
Rep 5	300	4.8	**5.2**	5.2	7.7	<-0.7	**>5.9**	-ve	**>7.7**
Rep 6	297	4.2	**5.3**	5.3	7.7	<-0.7	**>6.0**	-ve	**>7.7**
Rep 7	305	3.6	**5.3**	5.3	7.8	<-0.7	**>6.0**	+ve	**FAILED**

Pathogen Inactivation studies were performed to emulate the NHSBT experimental design and use full process

- Culture preparation, inoculation of the culture to the platelet units and INTERCEPT treatment , same as before.
- Post INTERCEPT Treatment, the full process as described in the IFU was used:
  - The treated platelets were transferred to the CAD container
    - Units on CAD for ~4 hours at 22°C on platelet shaker
  - At the end of CAD incubation, a 5mL sample was tested Post-CAD.
    - No colonies observed (>5.8 log inactivation)
  - Platelets were transferred to the storage containers and were incubated on a platelet shaker for 7 Days.
    - No colonies were observed (>7.7 log inactivation)

Rep #	Unit Volume (mL)	Plt. Dose (x10^11)	Control t=0	Control Pre t=0	Control Titer per Unit	Test	Log Red.	Day 7 growth	Log reduction per unit
Rep 8	285	4.1	5.2	**5.2**	7.7	<-0.5	**>5.8**	-ve	**>7.7**
Rep 9	283	4.4	5.2	**5.2**	7.6	<-0.5	**>5.7**	-ve	**>7.6**
Rep 10	329	3.9	5.1	**5.1**	7.6	<-0.7	**>5.8**	-ve	**>7.6**
Rep 11	307	4.0	5.1	**5.2**	7.7	<-0.7	**>5.9**	-ve	**>7.7**

Final Data Summary

Rep #	2 h RT Pre-incubation	Pre-UVA Titer	Post-UVA Titer	Log reduction	Log Reduction per Unit	7 D incubation	
						Post UVA	Full Process
1	NO	7.0	<-0.7	>7.0	NA	NA	NA
2	NO	7.0	<-0.7	>7.0	NA	NA	NA
3	Yes	6.8	<-0.7	>6.8	NA	NA	NA
4	NO	7.0	<-0.7	>7.0	NA	NA	NA
5	Yes	5.2	<-0.7	>5.9	>7.7	**No Growth**	NA
6	Yes	5.2	<-0.7	>6.0	>7.7	**No Growth**	NA
7	Yes	5.3	<-0.7	>6.0	7.8	**Growth (8.9 log)**	NA
8	Yes	5.2	<-0.5	>5.8	>7.7	NA	**No Growth**
9	Yes	5.2	<-0.5	>5.7	>7.6	NA	**No Growth**
10	Yes	5.1	<-0.7	>5.8	>7.6	NA	**No Growth**
11	Yes	5.2	<-0.7	>5.9	>7.7	NA	**No Growth**

Summary

- Follow up investigation of the inactivation of *S. marscecens* NHSBT, comprised eleven independent replicate experiments performed using apheresis platelets suspended in PAS.
- Four replicates were performed under conditions identical to ones used for regulatory claims **(>7.7 log**).
- Three replicates were performed extending the incubation to 7 days for platelets immediately post illumination **(2 replicates >7.7 log**); (**7.7>PI>5.9**)
- Four replicates were performed following the full INTERCEPT procedure with the CAD step and final transfer to the storage container, followed by storage for 7 days. (**>7.65**)
- The results from all 11 replicates are consistent with the previous claims for this bacterium in PC suspended in PAS **(>6.7 log)**.
- Inactivation failed when the challenge titer exceeded the claim
- The full process may provide additional robustness through the transfer steps