

# Slow Growing Bacterial Strains in Platelet Units Evade Detection with Culture Systems But Are Detected by the BacTx™ Assay, a Rapid Test for Bacterial Contamination of Platelet Units

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## Abstract (revised)

Existing technologies for detecting bacterial contamination in platelets are either very insensitive (e.g. glucose or pH strips) or very time consuming (automated culture.) Automated culture methods can take 24-72 hours or longer to detect slower growing bacterial strains, using up a significant portion of the shelf life of a platelet unit. For this reason, platelet bags are typically sampled 24 hours after collection for automated culture testing. The downside of such early sampling has been shown by the now defunct PASSPORT study. The BacT/ALERT culture system, when used as a release test, had a sensitivity of only 25.9%, and false positives outnumbered true positives by a factor of more than 3 to 1.

Gram positive bacteria, in particular *Staphylococcus aureus*, *S. epidermidis*, and other coagulase-negative *Staphylococci* account for the majority of cases of bacterial contamination of platelet units<sup>2-6</sup>. However, the slow growth kinetics of coagulase negative *Staphylococci* in platelets makes detection of these bacteria difficult or impossible if the bag is sampled early in its lifetime. The aim of this investigation was to compare the efficiency of detection of contaminated platelet units by traditional culture methods vs. a rapid point-of-use test, the BacTx™ assay.

Platelet units were spiked with 0.4-20 CFU/ml of gram-positive and gram-negative bacterial species, including Streps, Staphs and others. The concentration of bacteria in the platelet units was monitored every day for 3-5 days by standard culture/plating methods. Platelet bags were sampled and tested in the BacTx™ assay at each time point to determine sensitivity vs. concentration for each bacterial species.

Individual strains of a dozen common bacterial contaminants showed dramatically different growth kinetics in platelets. Fast-growing strains such as *Bacillus cereus* and *Klebsiella oxytoca* reached 10<sup>4</sup>-10<sup>7</sup> CFU/ml by 24 hours post inoculation. By contrast, inoculates of *Staphylococcus lugdunensis* and *Staphylococcus epidermidis* were reproducibly undetectable by *in vitro* culture methods for more than 2 days, reaching 800-10,000 CFU/ml 72 hours post-inoculation. In bags that did not auto-sterilize, the BacTx™ assay detected *S. lugdunensis* with 100% sensitivity at 8 x 10<sup>2</sup> CFU/ml 72 hours after inoculation, and *S. epidermidis* at 1-5 x 10<sup>3</sup> CFU/ml 48 hours after inoculation.

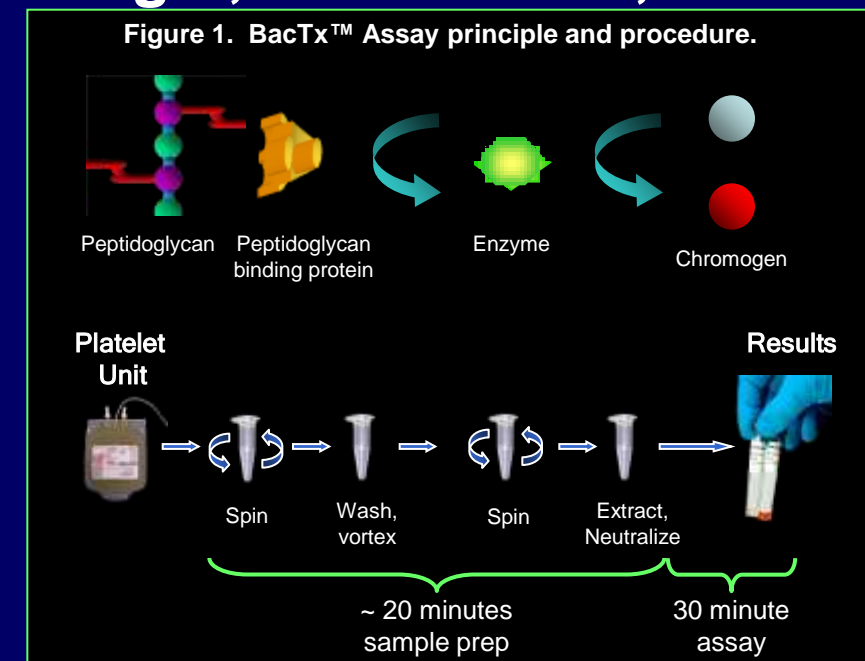
Current culture methods which require sampling the platelet bag 24 hours after collection have failed to detect contamination resulting from some slow-growing bacteria such as *S. epidermidis* and *S. lugdunensis*<sup>2-6</sup>. Such failures could be eliminated by relying on a test that can be performed directly on the platelet bag close to the time of transfusion as the primary test for platelet release, in place of culture. A consequence of such a change would be that sampling bags early in their lifetime becomes unnecessary once a real-time test result is available close to the time of transfusion. This paradigm could be implemented with a test such as BacTx™ which detects bacteria at less than 10<sup>4</sup> CFU/ml.

## Methods

**Bacteria.** *Staphylococcus lugdunensis* (ATCC 43809), *Staphylococcus epidermidis* (ATCC 49134), *Staphylococcus aureus* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Bacillus cereus* (ATCC 11778), *Streptococcus pyogenes* (ATCC 19615) and *Klebsiella oxytoca* (ATCC 13882) were cultured for testing. Bacterial cultures were grown in Trypticase Soy Broth or Brain Heart Infusion Broth (for *S. pyogenes*), diluted in phosphate buffered saline, and spiked into platelet bags using a syringe. Culture concentrations were determined by plating on Trypticase Soy Agar (TSA) plates or TSA Sheep Blood plates (*S. pyogenes*).

**Spiking platelets.** Random donor platelet units, either leukoreduced or non-leukoreduced, were obtained from Rhode Island Blood Center (Providence, RI) or Gulf Coast Regional Blood Center (Houston, TX), respectively. Platelet units were spiked with bacteria to generate initial titers in bags of 0.4-20 CFU/ml. The bags were then sampled at 24, 48, 72 and sometimes 96 hours for testing with the BacTx™ Assay, and aliquots were also removed from the bags for titering using standard *in vitro* culture methods. Sensitivity of detection was evaluated for each species spiked at various concentrations, indicated in the figures. The detection reaction was monitored kinetically either in a plate reader or in the BacTx™ Reader, a dedicated automated photometer.

**BacTx™ assay procedure** (Figure 1). Samples of 1 ml were sterilely removed from platelet bags for BacTx™ assay using a syringe. These were transferred to a microcentrifuge tube and centrifuged at 14,000 rpm for 4 minutes. 0.5 ml of Wash Buffer was added to resuspend the pellet, and the samples were auto-vortexed and heated for 4 minutes at 80°C in an Eppendorf ThermoMixer. Samples were again centrifuged at 14,000 rpm for 4 minutes, and 0.5 ml Extraction Reagent was added to the pellet, which was resuspended, auto-vortexed and heated at 80°C for 4 minutes. 0.5 ml of Neutralization Buffer was then added, and the resulting mixture was transferred to a reaction tube. Detection reagents were then added, and the absorbance of the resulting solution was read at 500 nm using the BacTx™ Reader.



Strain	Number of Spiked Bags Detected by Culture Methods		
	24 hours	48 hours	72 hours
<i>Bacillus cereus</i>	5/5	5/5	5/5
<i>Escherichia coli</i>	7/7	7/7	7/7
<i>Klebsiella pneumoniae</i>	5/7	7/7	7/7
<i>Klebsiella oxytoca</i>	8/8	8/8	8/8
<i>Pseudomonas aeruginosa</i>	5/6	6/6	6/6
<i>Staphylococcus aureus</i>	3/10	10/10	10/10
<i>Staphylococcus epidermidis</i>	0/10	6/10	10/10
<i>Staphylococcus lugdunensis</i>	0/15	11/15	15/15
<i>Streptococcus agalactiae</i>	0/2	2/2	2/2
<i>Streptococcus pyogenes</i>	4/8	6/8	8/8

Figure 2. Summary of bag spiking experiments. The number of bags detectable by culture methods at each time point is listed. For example, none of the bags spiked with *S. epidermidis* were detected by culture at 24 hours, 6 of 10 were detectable at 48 hours, and all 10 were detected at 72 hours.

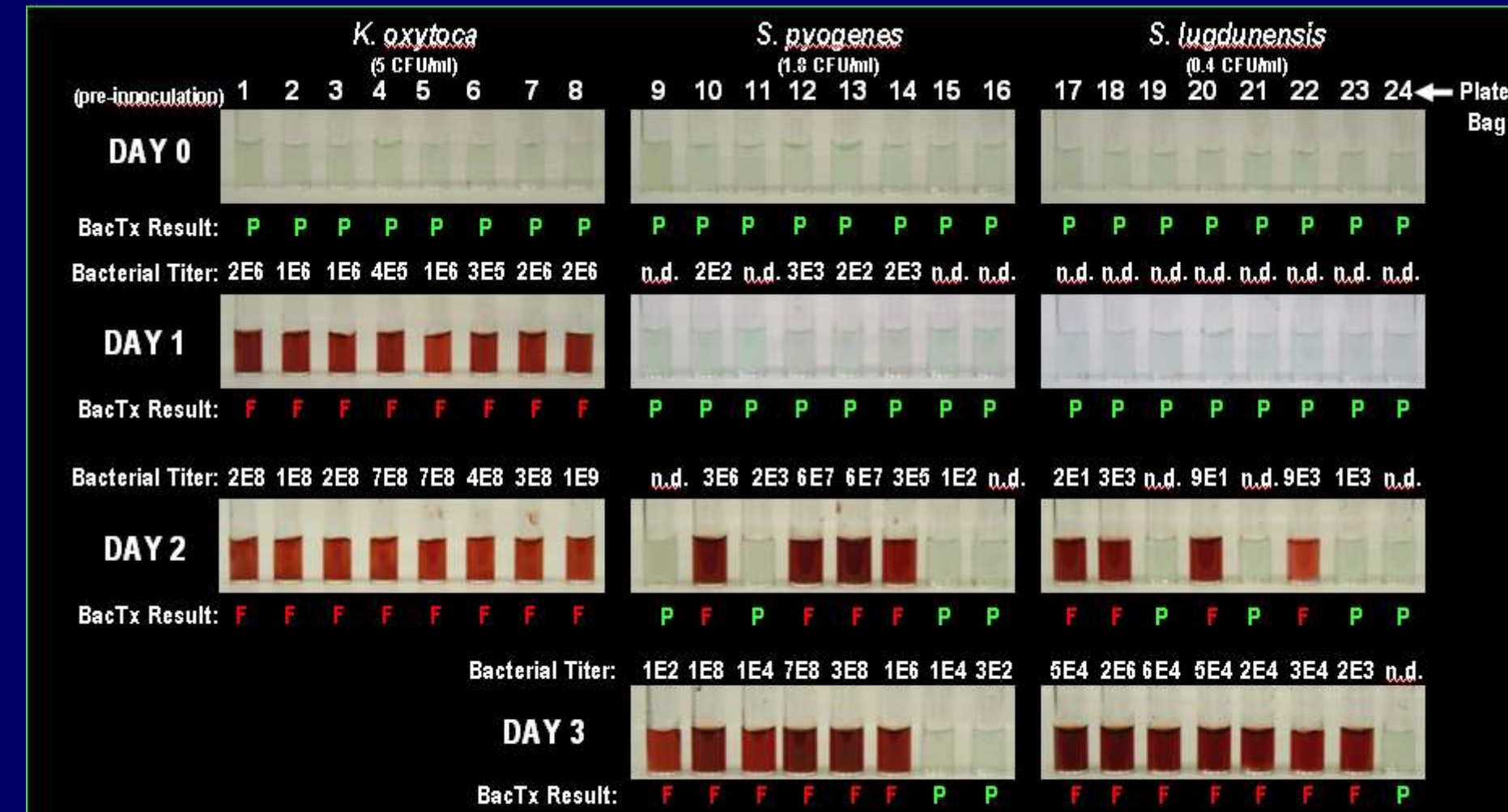


Figure 3. Bag spiking experiments with three bacterial species. Leukoreduced random donor units were spiked with the indicated concentrations of *Staphylococci*, and assayed on Day 0 (pre-inoculation), and on Days 1-3. In parallel, aliquots were removed from the bags and the titer of bacteria present in the bags was determined by culture methods. n.d. = not detectable. unk. = not determined. P = Pass, F = Fail.

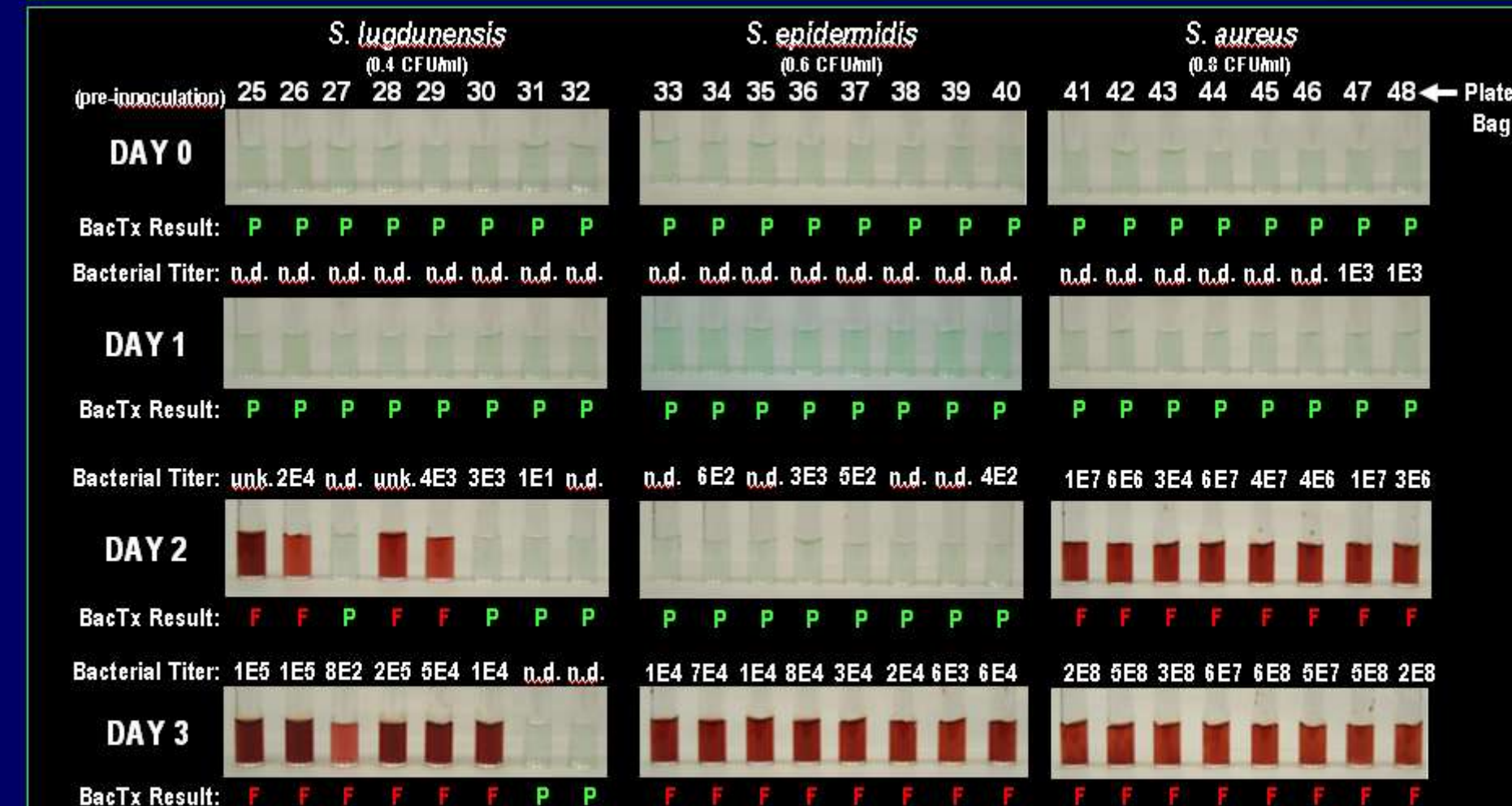


Figure 4. Bag spiking experiments with *Staphylococcal* species. Leukoreduced random donor units were spiked with the indicated concentrations of *Staphylococci*, and assayed on Day 0 (pre-inoculation), and on Days 1-3. In parallel, aliquots were removed from the bags and the titer of bacteria present in the bags was determined by culture methods. n.d. = not detectable. unk. = not determined. P = Pass, F = Fail.

## Results

Certain strains of coagulase negative *Staphylococcus* (e.g. *S. epidermidis*, *S. lugdunensis*, and *S. aureus*), are among the most common bacterial contaminants found in platelet units<sup>2,3</sup>. These same strains have been reported to have been missed by automated culture methods,<sup>4,5,6</sup> with dire consequences for patient safety. The BacTx™ assay detects all three of these strains at a sensitivity of 1-5 x 10<sup>3</sup> CFU/ml, sensitivities that meet or exceed the proposed AABB standards for a rapid test for bacterial contamination of platelets.<sup>7</sup>

In figures 3 and 4, we examined the growth kinetics of these strains, and others, when spiked at low levels into platelet bags. Not surprisingly, we found that these strains were frequently unable to be detected by standard culture methods for 24-48 hours post-inoculation, but at later times (e.g. 72 hours post-inoculation), were detectable by culture and by the BacTx™ assay. For example, *S. lugdunensis*, when spiked into platelet units at titers lower than 1 CFU/ml, was undetectable by culture at 24 hours in 13/13 bags that were subsequently shown not to have auto-sterilized. After 48 hours, culture was still unable to detect bacteria in 3/13 of the bags. Similarly, in the case of *S. epidermidis*, 8/8 spiked bags could not be detected by culture methods at 24 hours, and 4/8 bags were still culture negative at 48 hours. All 8 bags were subsequently culture positive and BacTx™ assay positive at 72 hours post-inoculation. Similar phenomenology has been observed for *S. aureus* (Fig. 4) as well as for certain species of *Streptococci*, e.g. *S. pyogenes* (Fig. 3).

Other strains reproducibly grow to high titers quite rapidly. For example, spiking platelets with low titers of *B. cereus* and *K. oxytoca* generated very high titers within 24 hours in every platelet bag tested. Our bag spiking kinetic data is summarized in Figure 2.

## Conclusions

Our data indicates that the inherent growth kinetics of certain common contaminants of blood products are intrinsically inconsistent with the current standard practice of sampling platelet bags early in their shelf life. The assumption that a negative culture result at 24 hours is predictive of sterility of the bag at later times is inconsistent with the growth properties of several critically important bacterial blood borne pathogens. Since these growth characteristics are innate properties of the bacteria themselves, improvement of culture-based detection methods will not solve the problem of bacterial contamination in platelets. In order to assure patient safety during transfusions, a paradigm shift in bacterial contamination testing is needed. Bacterial contamination testing will only be effective if it is conducted near the time of transfusion, by a test that provides a rapid and sensitive result.

The development of the rapid BacTx™ assay will permit blood banks and transfusion centers to test for bacterial contamination in a way that optimally detects contaminated platelet units. The BacTx™ assay represents a significant advancement over culture methods, which only provide information about the condition of platelet units one or several days in the past. The failure of the PASSPORT study<sup>1</sup> highlights the importance of shifting bacterial contamination testing to near the time of transfusion. It is a challenge for the blood banking industry to incorporate changes in the temporal sequence of bacterial contamination testing. However, the documented failure of culture based methods to detect contaminated platelet units early in the life of platelet units has generated a situation where patient safety is constantly at risk.

## References

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