ISBT Science Series (2006) **1**, 59–63

ORIGINAL PAPER **2PS-05-01**

The reliability of bacterial detection in platelets

J. P. AuBuchon

E. Elizabeth French Professor and Chair of Pathology and Professor of Medicine, Dartmouth-Hitchcock Medical Center, One Medical Center Drive, Lebanon, NH, USA

Introduction

Growing recognition of the frequency of bacterial contamination of platelet units and of the clinical consequences of that contamination have prompted blood collection organizations in many countries to implement efforts to address this risk. Most facilities have adopted bacterial culture as the means to detect this contamination, although other techniques, including fluorescent and electrostatic detection, have been described. Microscopy and surrogate techniques have also been employed to test individual platelet units derived from whole blood. The general perception of blood bankers is that these measures, often taken in concert with improved skin antisepsis techniques and diversion of the initial volume of blood collected, have reduced the frequency of clinically significant bacterial contaminations in platelet units. However, quantification of this improvement has been difficult to obtain.

Infectious disease testing systems designed for application to donated blood have evolved significantly over the last two decades. Viruses that were once detectable only through the immunologic response they caused can now be detected directly at remarkably low levels. Coupled with pre-donation screening of prospective donors and the relatively low incidence of new (i.e. low-viraemia) infections among donors, nucleic acid amplification testing (NAT) affords a level of safety unimaginable just a few years ago. Bacterial contamination, however, poses a different set of challenges and opportunities. While a donor verified as having less than the usual NAT threshold of several hundred virions per ml is almost always truly not infected, a single bacterium contaminating a newly collected unit of blood could multiply during storage and lead to the rapid demise of the recipient. Thus storage time is both an ally and an opponent: Sampling can be delayed until bacterial proliferation has generated a concentration that can readily be detected through a small aliquot, but, especially for platelets, this time cuts into the already brief storage period, potentially creating logistic difficulties [1]. Unlike with viral contamination, this multiplication during storage could be used to allow rapid but less sensitive testing to be performed shortly before issuing the unit for transfusion with the assumption that any pathogenic organisms would have multiplied to detectable levels during the storage period.

Sensitivity of bacterial detection methods may be considered in terms of analytic sensitivity and clinical sensitivity (Table 1). *Analytic sensitivity* represents the likelihood of contamination being detected and is a product of the initial inoculum (usually below 10 CFU/ml and probably often below 1 CFU/ml), the time (and temperature) between collection and sampling, and the size of the inoculum in addition to the ability of the device to the support of growth of organisms. This sensitivity may be different for different organisms depending on their growth characteristics. The presence of bacteriocidal phagocytic leucocytes and/or antibodies and complement in the collected blood may alter not only the speed at which contamination becomes detectable but whether any of the initial inoculum remains viable to proliferate. The question comes down to: Assuming that the organism will grow and be detected in the test system, what is the probability that the (relatively small) sample will demonstrate the presence of bacteria if the unit has been contaminated?

In surrogate, rather than culture systems, the growth of organisms must be detected through a change that they cause in their environment, such as a drop in glucose concentration or pH, and thus analytic sensitivity also reflects the ability to distinguish what the signal from a contaminated unit vs. that parameter in a sterile unit [2,3]. While any detection system has a 'background' from which the 'signal' must be distinguished, this background is more problematic for surrogate systems since it changes over (storage) time and between units (that may have different platelet and leucocyte concentrations).

Clinical sensitivity derives from the implication that not all bacteria are equally likely to multiply in a contaminated unit to a concentration that represents an inoculum that may result in recipient morbidity or mortality. While no one would knowingly transfuse a unit that is not sterile, we know from decades of experience that many organisms do not have the pathogenic capacity or growth characteristics to develop clinically significant inocula during the time period of platelet storage. The ability of the recipient to respond to infections of small inocula of organisms that are of low pathogenicity

Table 1 Determinants of sensitivity of bacterial detection systems

Analytic sensitivity	Clinical sensitivity
Persistence to time of sampling	Pathogenicity of organism
Sample size	Growth rate in system vs. unit
Time from collection to sampling	Time from sampling to detection
Growth potential during holding period	Frequency of pathogen among contaminants
Temperature	
Replication time	
Ability of system to support growth	Effectiveness of system to interdict 'signal-positive' units before transfusion
Distinction between sterile and contaminated units	

also is part of this calculus. The 'bottom line' is that while 'perfect detection' of all bacteria may provide comforting assurance of sterility, it is probably not necessary and may yield undesirable consequences in terms of cost and logistic complications.

Analytic sensitivity

Documenting the analytic sensitivity of bacterial detection systems has proven very difficult. Prior to their implementation or regulatory approval for use in blood banking, most validation studies have focused on the ability of the device and the protocol for its use to detect the presence of bacteria that have been intentionally spiked into (sterile) units [4–6]. This approach defines the ability of a certain strain of an organism to grow up from a defined initial concentration (usually around 1 CFU/ml but perhaps as high as 10 CFU/ml) to a detectable concentration. This approach to validation may not fully reflect reality in that the organism was probably already growing in log phase prior to spiking and the initial concentration may have been unrealistically high. Changes in the capabilities of the organism following successive passages in an *in vitro* growth medium may also affect its growth characteristics in a unit of platelets. On the other hand, the unit's plasma may have antibodies against the organism that entirely neutralizes the inoculum or reduces it significantly so that the time to detection is longer than might have been found in other units. For reasons such as this, although the potential applicability of a system to the testing of platelets can be mirrored in spiking experiments, analytic sensitivity is better measured in a 'real world' trial.

The analytic sensitivity of the system in a real world trial would be documented by determining its ability to identify naturally occurring contaminations. Such a trial would require retesting of negative units via a 'gold standard', usually defined as a culture technique performed later in storage at a time when any contaminating inoculum would certainly have multiplied to the point of a guaranteed detectability. Given the relatively low frequency of contamination and the low frequency of false-negative initial outputs of the detection system, such studies must be very large. For example, in the US, the FDA has established that an acceptable residual risk of undetected bacterial contamination that would support extension of storage to 7 days would be 1 per 10 000 units with an upper bound of the 95% confidence interval as 1 per 5000 units; their calculations placed the required sample size as 50 000 units, a daunting sample size indeed!

(As part of approval of storage for 7 rather than 5 days, a study is currently underway in the US to define whether the FDA's risk criteria are being met. Units are cultured 24–36 h after collection via a defined method (4 ml into each of two BacT/Alert (Biomerieux, Durham, NC) bottles, one for aerobic culturing, the other for anaerobic culturing). Units that outdate after 7 days of storage are then re-cultured to provide the definitive statement regarding whether they were truly free of bacterial contamination. The study is expected to take several years to complete.

Several studies have already been published that shed some light on this issue. Rock and colleagues cultured 12 062 platelet units derived form units of whole blood on Day 1 of storage, finding four positive for a rate of approximately 1/3000, a common finding [7]. Pools of six units were created immediately before release for transfusion, and a culture was performed on the pool. One culture from 2201 pools was positive, indicating that the risk of a contaminated units slipping through the initial culture was around 1/12 500. Similarly, Larsen *et al.* performed 36 896 cultures on buffy coat pools (83%) and apheresis platelet units (17%) at 31–61 h after collection [8]. Again, about 1/3000 units were found to be contaminated on the initial culture. On Day 5–7, a repeat culture was performed on 1061 pools (or units), and two were confirmed as being contaminated. This suggests a much higher residual risk, perhaps indicating that 1 in approximately 500 transfusions might harbour bacteria. (Although pooling before culturing would theoretically reduce analytic sensitivity, this study's findings are particularly disconcerting when it is realized that the pre-sampling holding time was significantly longer than the usual holding

period of ~24 h that is used.) None of the reported experience with culturing early in storage have yet matched the higher contamination rate seen with sterility culturing at outdate of around 2/1000 [9].

At least five clinical 'breakthrough cases' have been reported despite bacterial culturing of platelet units [10–13]. Given the limitations of analytic sensitivity as described above, this is not surprising. While some of these have caused only mild to moderate recipient morbidity, others have been fatal. The American Red Cross Blood Services has received reports from hospitals that would place the residual risk of post-transfusion sepsis at 1/74 000 units transfused and the risk of fatality due to bacterial contamination of platelets at 1/650 000 units (R Benjamin, C Fang, personal communication, April 2006). Of course, these may be underestimates given that not all transfusion-related infections are appreciated for their aetiology [14,15] nor reported to the blood supplier, but this experience would indicate a significant improvement over no culturing [16,17].

Using 'surrogate' detection systems with more limited analytic sensitivity might be expected to yield a greater proportion of 'false negatives'. (Some centres have felt that culture-based detection could not be applied to individual units of whole-blood derived platelets in blood banking systems where pre-storage pooling was not allowed.) For example, using a pH meter to identify production of organic acids by contaminating bacteria in whole blood-derived platelet units identified 405 of 37 060 with an abnormally low pH, four of which were then shown to be culture-positive [18]. The frequency of identification of contaminated units (approximately 0·11/1000) is half the rate described during the same time period by US blood centres using a culture-based detection method [11,19] and one-third the usually reported rate [20]. Although almost all apheresis platelet units generated in the US are cultured by the collecting blood centre [1], 20% of platelet transfusions supplied as whole blood-derived units are cultured in only a minority [13%] of hospitals and instead, most frequently, have their pH or glucose concentration checked either by instrument or by urine dipsticks [21]. Although such approaches clearly do not provide optimal sensitivity for bacterial detection, some contaminated units are interdicted.

Several steps could be considered to improve analytic sensitivity. A longer pre-sampling time might be expected to be particularly efficacious since bacterial concentration would be increasing exponentially through this (additional) time. Indeed, our experience with culturing on Day 2 has been the finding of a much higher rate of confirmed contamination (0·5–1/1000 units) than that found in the US using essentially the same protocol but with culturing performed on Day 1 (0·2/1000 units) [22]. However, extending this time poses additional logistic burdens on a system that already is affected to some degree by bacterial detection [1]. Increasing the volume cultured would also increase theoretical sensitivity although only arithmetically. However, one direct trial of this approach, increasing the cultured volume from 2 to 8 ml, did not shorten the time to detection of spiked samples and thus may not achieve the theoretical benefit of increased sensitivity as well [23]. Some have focused attention on anaerobic bacteria and advocated for the utilization of an anaerobic culture as well as an aerobic one. The platelet storage environment, of course, is a decidedly aerobic one, and how an anaerobe could multiply to dangerous levels is unclear. Although there are several case reports of *Clostridium* in platelet units causing death [24] these must have been *facultatively aerobic* strains. It is not known whether some would have been detected in current systems that depend on aerobic cultures, but at least one failed to grow aerobically. (The contribution of this genus to platelet contamination risks is very small, however.) Those centres that utilize anaerobic cultures report the not-infrequent detection of *Propionibacterium* spp. This skin contaminant is thought to be of extremely low pathogenicity, although infection of prosthetic cardiac valves has been seen (but not through platelet transfusions) [25]. Several reports of earlier detection of aerobic (but facultatively anaerobic) organisms through use of anaerobic bottles may reflect a combination of strain or inoculation serendipity as well as the composition of the culture media [26]. Thus while the contribution of the addition of anaerobic cultures may improve analytic sensitivity, their contribution to clinical sensitivity remains open for debate. In the USA, the largest increase in sensitivity would occur with implementation of effective detection techniques for whole blood-derived platelets.

Clinical sensitivity

Clinical significance is determined by the ability of the detection system to identify those units contaminated with organisms the frequency and pathogenicity of which combine to yield the greatest potential for morbidity and mortality. This concept has several components.

One aspect of clinical sensitivity pertains to the rapidity with which a signal is given that the unit should be interdicted. Such a quarantine is easiest to accomplish while the unit is still in the blood centre (that is, before it has been released to a hospital transfusion service). Since culture-based systems depend on multiplication both in the unit and in the sample that is taken from it, 'time to detection' becomes an important factor. With cultures taken on Day 1 or 2 and placed in an automated system, most signals from truly contaminated units occur within 20–24 h [13,17,20] As a result, some blood centres have opted to retain units in inventory for 12–24 h before releasing them to hospitals as 'negative-to-date', but this approach has contributed to the reports of culturing affecting the availability of platelets in some circumstances [1]. The eBDS culture system (Pall Medical, Glen Cove, NY)

has a defined analysis time, so a 'late report' and retrieval of a released unit would not occur [27]. Similarly, the ScanSystem (HemoSystems, Marseille, France) or polymerase chain reaction-based testing yields a result in a short time after a pre-sampling holding period, making the endpoint of the detection protocol clearer [28–30]. A 'late signal' in an automated culture system is most likely with a 'slow grower'. As these kinetics would also be evident in the unit itself and since the rapidly proliferating (often gram negative) contaminants are the ones most likely to cause post-transfusion mortality [8,31], units transfused before the signal of growth from the automated detection system usually are not associated with untoward clinical outcomes – or even any clinical evidence of bacterial transmission [10]. However, avoidance of transmission of even small amounts of potentially pathogenic bacteria may be advisable when the recipients are immunosuppressed and neutropenic.

A key unanswered question is: 'How many bacteria are dangerous'? The two most applicable answers are: (1) it depends on the circumstances; and (2) we don't really know. Those procedures intended to be performed relatively earlier in storage (e.g. ScanSystem, and the peptidoglycan detection system under development by Immunetics, Watertown, MA [32]) would require exquisite sensitivity since a prolonged period of storage may follow the point of testing. Those systems intended for pre-transfusion testing, that is, within a few minutes to hours of transfusion (such as the lateral flow immunologic detection device developed by Verax Biomedical, Worcester, MA [33]) may be very useful despite their analytic sensitivity being several orders of magnitude less than that of culture-based systems. For example, most bacteria that could multiply in platelets at room temperature would likely generate a concentration well above an analytic sensitivity limit of 10^3 or 10^4/ml by Day 3 of storage, and the contaminated unit would be prevented from transfusion. The possibility would remain, however, that a small initial inoculum of a slow-growing organism might not have reached that threshold by the time of (early) transfusion, and a contaminated unit would be transfused. The chance of that occurring, given the average age of platelets at transfusion and the distribution of organisms contaminating platelets and their growth kinetics, appears to be so low that even that the FDA's target of a residual risk of not greater than 1/10 000 units would be met. (Carl Shafer, personal communication, April 2006) Furthermore, fatal septic events after platelet transfusion appear to be skewed towards shorter storage periods suggesting that the rapid-growing organisms – the ones most likely *not* to escape detection with a pre-transfusion detection system – would be prevented from wreaking their havoc [8]. We would still be faced with the prospect of possibly transfusing a lower amount of a slow-growing organism on rare occasion, an event that already occurs with apparently little clinical consequence.

Where do we go from here?

Those who have been fomenting action for many years to reduce the risk of bacterial contamination are delighted at the efforts of so many blood centres to increase the safety of platelet transfusions by taking steps to prevent contamination and to detect that which may be occurring. In the spirit of continuous quality improvement as well as dedication to the welfare of our patients, we should seek to improve these efforts until bacterial contamination deaths are as rare as HIV transmission in most developed countries.

How can we accomplish that? Steps to improve analytic sensitivity were outlined above, but the low initial inocula and brief platelet storage periods combine to work against efforts to achieve exquisite sensitivity. Although pre-transfusion detection systems under development may provide useful clinical sensitivity, achievement of sterility will require implementation of pathogen inactivation. Here, the low initial inocula encountered are readily handled by either amotosalen and ultraviolet light or riboflavin and ultraviolet light within a day or so of collection [34–36]. The concern about the resistance of spores to inactivation techniques can be obviated by requiring at least a several-hour hold before applying the treatment in order to allow the spores to convert to the (susceptible) vegetative phase. Several European centres have implemented pathogen inactivation techniques and dropped bacterial culturing. The advantage of assured bacterial sterility afforded by pathogen inactivation should be considered along with its ability to address the risks of other pathogens and its potential to allow avoidance of cumbersome culture techniques.

We have the capability to reduce the risks associated with bacterial contamination by the same degree as we have with HIV and HCV, and we should take the opportunity to apply these improvements.

References

1 Silva MA, Gregory KR, Carr-Greer MA, Holmberg JA, Kuehnert MJ, Brecher ME for the Task Force: Summary of the AABB Interorganizational Task Force on Bacterial Contamination of Platelets: Fall 2004 impact survey. *Transfusion* 2006; **46**:636–641

2 Burstain JM, Brecher ME, Workman K, Foster M, Faber GH, Mair D: Rapid identification of bacterially contaminated platelets using reagent strips: glucose and pH analysis as markers of bacterial metabolism. *Transfusion* 1997; **37**:255–258

3 Wagner SJ, Robinette D: Evaluation of swirling, pH, and glucose tests for the detection of bacterial contamination in platelet concentrates. *Transfusion* 1996; **36**:989–993

4 Brecher ME, Means N, Jere CS, Heath D, Rothenberg S, Stutzman LC: Evaluation of an automated culture system for detecting bacterial contamination of platelets: an analysis with 15 contaminating organisms. *Transfusion* 2001; **41**:477–482

5 Brecher ME, Heath D, Hay S, Rothenberg S, Stutzman LC: Evaluation of a new generation culture bottle using an automated bacterial culture system for detecting nine common contaminating

organisms found in platelet components. *Transfusion* 2002; **42**:774–779

6 Brecher ME, Hay SN, Rothenberg SJ: Evaluation of a new generation of plastic culture bottles with an automated microbial detection system for nine common contaminating organisms found in PLT components. *Transfusion* 2004; **44**:359–363

7 Rock G, Neurath D, Toye B, Sutton D, Giulivi A, Bormanis J, Olberg B, Holme S, Wenz B, Ortolano G, Nelson E: The use of a bacteria detection system to evaluate bacterial contamination in PLT concentrates. *Transfusion* 2004; **44**:337–342

8 Larsen CP, Ezligini F, Hermansen NO, Kjeldsen-Kragh J: Six years' experience of using the BacT/ALERT system to screen all platelet concentrates, and additional testing of outdated platelet concentrates to estimate the frequency of false-negative results. *Vox Sang* 2005; **88**:93–97

9 Walther-Wenke G, Doerner R, Montag T, Greiss O, Hornei B, Knels R, Strobel J, Volkers P, Daubener W: Working party on Bacteria Safety in Transfusion Medicine of the Advisory Board of the German Ministry of Health (Arbeitskreis Blut), Berlin, Germany. Bacterial contamination of platelet concentrates prepared by different methods: results of standardized sterility testing in Germany. *Vox Sang* 2006; **90**:177–182

10 Te Boekhorst PAW, Beckers EAM, Vos MC, Vermeij H, van Rhenen DJ: Clinical significance of bacteriologic screening in platelet concentrates. *Transfusion* 2005; **45**:514–519

11 Centers for Disase Control and Prevention: Fatal bacterial infections associated with platelet transfusions – United States, 2004. *Morb Mort Weekly Rep* 2005; **54**:167–169

12 Benjamin RJ, Mintz PD: Bacterial detection and extended platelet storage: the next step forward. *Transfusion* 2005; **45**:1832–1834

13 Fang CTR, Chambers LA, Kennedy J, Strupp A, Fucci MC, Janas JA, Tang Y, Hapip CA, Lawrence TB, Dodd RY: Detection of bacterial contamination in apheresis platelet products: American Red Cross experience, 2004. *Transfusion* 2005; **45**:1845–1852

14 Chiu EKW, Yuen KY, Lie AKW, Liang R, Lau YL, Lee ACW, Kwong YL, Wong S, Ng MH, Chan TK: A prospective study of symptomatic bacteremia following platelet transfusion and of its management. *Transfusion* 1994; **34**:950–954

15 Blajchman MA, Beckers EA, Dickmeiss E, Lin L, Moore G, Muylle L: Bacterial detection of platelets: current problems and possible resolutions. *Transfus Med Rev* 2005; **19**:259–272

16 Ness PM, Braine HG, King K, Barrasso C, Kickler T, Fuller A, Blades N: Single-donor platelets reduce the risk of septic platelet transfusion reactions. *Transfusion* 2001; **41**:857–861

17 Andreu G, Morel P, Forestier F, Debeir J, Rebibo D, Janvier G, Hervé P: Hemovigilance network in France: organization and analysis of immediate transfusion incident reports from 1994 to 1998. *Transfusion* 2002; **42**:1356–1364

18 Yazer MH, Triulzi DJ: Use of a pH meter for bacterial screening of whole blood platelets. *Transfusion* 2005; **45**:1133–1137

19 Kleinman S, Kamel H, Harpool D, Vanderpool S, Custer B, Wiltbank T, Nguyen KA: Two year experience with aerobic culturing of apheresis and whole blood derived platelets. *Transfusion*, in press

20 Yomtovian R: Bacterial contamination of blood: lessons from the past and road map for the future. *Transfusion* 2004; **44**:450–460

21 College of American Pathologists: J-B proficiency testing survey supplemental questions, Northfield, IL 2004

22 AuBuchon JP, Cooper LK, Leach MF, Zuaro DE, Schwartzman JD: Experience with universal bacterial culturing to detect contamination of pheresis platelet units in a hospital transfusion service. *Transfusion* 2002; **42**:855–861

23 Fang CT, Hapip CA: Effects of platelet sample volume on bacteria detection time in the BacT/Alert system. *Transfusion* 2005; **45**:53A

24 McDonald CP, Hartley S, Orchard K, Hughes G, Brett MM, Hewitt PE, Barbara JA: Fatal *Clostridium perfringens* sepsis from a pooled platelet transfusion. *Transfus Med* 1998; **8**:19–22

25 Pan SC, Wang JT, Hsueh PR, Chang SC: Endocarditis caused by Propionibacterium acnes: an easily ignored pathogen. *J Infect* 2005; **51**:e229–31

26 Brecher ME, Hay SN, Rothenberg SJ: Validation of BacT/ALERT plastic culture bottles for use in testing of whole-blood-derived leukoreduced platelet-rich-plasma-derived platelets. *Transfusion* 2004; **44**:1174–1178

27 Holme S, McAlister MB, Ortolano GA, Chong C, Cortus MA, Jacobs MR, Yomtovian R, Freundlich LF, Wenz B: Enhancement of a culture-based bacterial detection system (eBDS) for platelet products based on measurement of oxygen consumption. *Transfusion* 2005; **45**:984–993

28 Mohammadi T, Piertsz RNI, Vandenbroucke-Grauls CMJE, Savelkoul PHM, Reesink HW: Detection of bacteria in platelet concentrates: comparison of broad-range real-time 16S rDNA polymerase chain reaction and automated culturing. *Transfusion* 2005; **45**:731–736

29 Jacobs MR, Bajaksouzian S, Windau A, Palavecino EL, Yomtovian R: Evaluation of the Scansystem method for detection of bacterially contaminated platelets. *Transfusion* 2005; **45**:265–269

30 Schmidt M, Weis C, Heck J, Montag T, Nicol SB, Hourfar MK, Schaefer V, Sireis W, Roth WK, Seifried E: Optimized Scansystem platelet kit for bacterial detection with enhanced sensitivity: detection within 24 h after spiking. *Vox Sang* 2005; **89**:135–139

31 Roth VR, Kuehnert MJ, Haley NR, Gregory KR, Schreiber GB, Arduino MJ, Holt SC, Carson LA, Elder KV, Jarvis WR: Evaluation of a reporting system for bacterial contamination of blood components in the United States. *Transfusion* 2001; **41**:1486–1492

32 Kirby C, Beausang L, Levin A, Kovalenko V: A rapid assay for the detection of bacteria in platelet units. *Transfusion* 2005; **45**:53A–54A

33 Hall J, Litwak G, Lajoie C: Detection of culture-positive platelet units by testing in-date and out-date platelet concentrates with the platelet PGD test. *Transfusion* 2004; **44**:48A–49A

34 Lin L, Dikeman R, Molini B, Lukehart SA, Lane R, Dupuis K, Metzel P, Corash L: Photochemical treatment of platelet concentrates with amotosalen and long-wavelength ultraviolet light inactivates a broad spectrum of pathogenic bacteria. *Transfusion* 2004; **44**:1496–1504

35 Ruane PH, Edrich R, Gampp D, Keil SD, Leonard RL, Goodrich RP: Photochemical inactivation of selected viruses and bacteria in platelet concentrates using riboflavin and light. *Transfusion* 2004; **44**:877–885

36 Nussbaumer W, Allersdorfer D, Grabmer C, Kilga S, Rheinschmidt M, Corash L, Lin L, Schoenitzer D, Lass-Floerl C: Comparison of BacTAlert and INTERCEPT in preventing the release of PLT units containing low numbers of viable bacteria. *Transfusion* 2005; **45**:52A–53A

