Methods for Determining Bacterial Contamination of Platelet Products

Subjects: Microbiology

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A wide variety of direct and indirect methods have been used to test platelets for the presence of bacterial contamination, and those that are in current use will be discussed. Tests performed on platelet products have been defined by the recent US FDA guidance document on this subject as primary and secondary. Primary testing is the initial bacterial detection test, usually by culture, performed following collection and before release of products for transfusion. Secondary testing is any additional test to detect bacteria in a platelet unit that showed no bacterial contamination upon primary testing. Secondary testing can be by culture or rapid testing methods.

platelet safety | bacterial contamination | platelet culture methods

1. Culture

1.1. Direct Culture

Small volumes of liquids can be inoculated directly onto agar plates, which are then incubated in a variety of atmospheres and temperatures [1]. Direct culture of platelet products is typically performed by inoculating 0.1 mL volumes onto blood agar plates, which are incubated in a 5% CO_2 atmosphere for up to 48 h. Anaerobic cultures can also be performed by incubating a second plate in an anaerobic atmosphere. The bacterial load of the specimen can be calculated from the number of colonies that grow, and serial 10-fold dilutions of a positive sample can be cultured to determine the bacterial load if the undiluted culture has too many colonies to count accurately. Direct culture is indicated when expected bacterial loads are higher than the sensitivity of direct plate culture (10–100 CFU/mL [2]), for example at time of issue of platelet products.

1.2. Enriched Culture

When expected bacterial loads are low, for example close to time of collection of platelet products, higher volumes of the product need to be cultured using broth culture media as is done for blood cultures. Three commercially available systems are currently available for performing enriched cultures using bottles containing suitable culture media and atmospheres for aerobic and anaerobic culture—BacT/ALERT (bioMérieux, Marcy-l'Étoile, France), Bactec (BD Biosciences, San Jose, CA, USA) and VersaTREK (ThermoFisher, Waltham, MA, USA). Automated instruments used to test bottles are: BacT/ALERT 3D and BacT/ALERT VIRTUO, Bactec FX system and VersaTREK Automated Microbial Detection System [3][4]. Culture bottles can be inoculated with up to 10 mL per bottle. Bottles are incubated in instruments at 35 °C and monitored for colorimetric changes in pH sensors in the

bottles as a result of CO_2 produced by growing microorganisms or pressure changes in the headspace secondary to gas consumption or production [5]. Bacterial counts at the time of detection by the three automated culture systems ranged from 10^7 to 10^{10} CFU/mL, with the majority being 10^8 to 10^9 CFU/mL [4].

The entire contents of a platelet unit can be cultured using an enrichment culture method, which is particularly useful in evaluating the efficacy of pathogen reduction [6].

Sensitivity of culture bottles is around 1 CFU/mL [2]. A report from a period where 8 mL volumes of platelet collections or pools were cultured using aerobic BacT/ALERT bottles estimated that there are 19 collections with low counts of dormant bacteria that are not readily detected by early BacT/ALERT culture for every confirmed positive contaminated collection detected [7]. The sensitivity of primary culture has been estimated to be 31% [8], highlighting the limitations of primary culture to detect bacterial contamination in platelet concentrates.

2. Rapid Testing

While many methods for rapid testing have been developed, only one, the PGD*prime* Test (Verax Biomedical, Marlborough, MA, USA) is currently commercially available in some countries [9]. This test is a rapid, lateral-flow, qualitative immunoassay for the detection of aerobic and anaerobic Gram-positive and Gram-negative bacteria and is used on the day of transfusion to extend the storage of leukocyte-reduced apheresis platelets in plasma from 5 to 7 days. The test detects a broad range of pathogenic bacteria at loads of 1.9×10^3 to 2.5×10^6 CFU/mL, and its sensitivity and specificity as well as ease-of-use have recently been improved over the original PGD Test [10].

In an earlier study using the original PGD Test, the test detected bacterial contamination in 9 of 27,620 platelet doses (326 per million) released as negative by primary culture [11]. The specificity of the PGD*prime* Test in a study of 3800 platelet components of all US platelet product types (except pathogen-reduced) showed no false-positives (100% specificity, with a lower 1-sided 95% confidence limit of 99.9%) [12]. Use of the test to extend storage from 5 to 7 days has been demonstrated to significantly reduce outdating, with more than 1.4 million PGD devices shipped to users without any fatal septic reactions resulting from the transfusion of a PGD-negative platelet product.

3. Test Interpretations

AABB Bulletin #04-07, Actions Following an Initial Positive Test for Possible Bacterial Contamination of a Platelet Unit, issued in October 2004 and updated in June 2022, provides interpretations of tests used to detect bacterial contamination of platelet products, methods for confirmatory testing of initial positive results and definitions for the interpretation of findings [13]. This bulletin also discusses the management of other co-components associated with the same donation and provides guidance to address when a positive test result is encountered only after transfusion of the unit, and when a recipient develops culture-proven posttransfusion sepsis. In this bulletin, a “test” is defined as any method implemented to detect bacterial contamination of platelet products, whether by a culture-based or non-culture-based method. Tests can be interpreted as initial positive, true positive, false positive, indeterminate or false negative. These interpretations apply in any circumstances, including (1) when the

component has not been issued, (2) when the component has been issued and transfused, and (3) when the component has been issued based on a negative initial test and the recipient developed posttransfusion sepsis confirmed by a positive culture.

1. Initial positive

Positive or abnormal (out-of-range) initial test. When applied to automated instrument systems monitoring culture bottles, an instrument signal indication positivity by the detection method used by the instrument indicates an initial positive result. When applied to rapid tests, this means that an initial positive result was obtained.

2. **True positive**

Positive on both the initial test and a confirmatory test. The confirmatory test must be culture-based and be performed on a different sample than the culture bottle or sample used for the initial test. The sample source for the confirmatory test is typically the original platelet component, which can be tested by the same culture method used to test the original specimen. A subculture of the initial positive culture bottle is not an adequate sample for this purpose. If a sample is not available for confirmatory testing because the unit has been transfused, posttransfusion sepsis in the recipient verified by positive culture is also defined as a true positive. These definitions imply, although they do not definitively state this, that a true positive requires that the bacterial species cultured from the confirmatory culture or the recipient is the same as the species found in the initial positive culture.

3. **False positive**

A false positive is defined as a positive initial test, negative confirmatory test **and no** clinical or microbiological evidence of posttransfusion sepsis in the recipient. False-positive results may occur for several reasons, including contamination during inoculation and by machine or reading error. All are included under this heading for the purposes of these definitions. There are, however, other reasons for false positives that are not included in the AABB false positive definition; these include (1) the presence of low inocula of bacterial species that do not grow in platelet products such as *Cutibacterium* (*Propionibacterium*) *acnes*, where only some of multiple cultures performed at the same time point may be positive [14], and (2) auto-sterilization of the platelet unit resulting in negative confirmatory cultures. Auto-sterilization has been described with coagulase-negative staphylococci [15] and *Bordetella holmesii* [16].

4. **Indeterminate**

A positive initial test with either no confirmatory test performed or confirmatory test results that could not be interpreted is interpreted as indeterminate. A negative initial test with no confirmatory test performed and recipient shows evidence of posttransfusion sepsis is also interpreted as indeterminate; in this definition evidence of sepsis is presumed to be clinical as microbiological evidence of sepsis is interpreted as a false negative initial test. Other combinations of component and recipient results in situations where the component has been transfused are also interpreted as indeterminate.

5. **False negative**

A negative initial test but the remaining available sample of the unit is positive by confirmatory test after the component has been transfused to a recipient who develops culture-proven posttransfusion sepsis. The same microorganism should be isolated from the component and the recipient. To the extent possible, other sources of bacteremia (e.g., infected indwelling catheter) should be excluded.

6. **True negative**

As part of an investigation of reported posttransfusion sepsis, the unit tests negative on the initial test and the remaining available sample of the unit is negative by confirmatory test.

In clinical practice, the vast majority platelet units with negative initial tests are released after a holding period and then transfused with no transfusion reaction, with the initial test remaining negative for the shelf-life of the unit. The interpretation of these circumstances based on the above AABB definitions is indeterminate as a negative confirmatory culture is required to meet the definition of a true negative.

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