Rapid Assay for Bacteria Detection in Platelets

Edward Burns, MD, Ding W. Wu, MD, David Kardon, MD, Min-Guang Wang, MD, John McKitrick, PhD
Albert Einstein College of Medicine and Montefiore Medical Center, New York, NY

DOI: 10.1309/03YXJKQUPUKJ3G9W

Sepsis due to platelets contaminated by bacteria is often caused by *Staphylococcus epidermidis* and diptheroids.

We developed a rapid latex agglutination test for the detection of *S. epidermidis* contamination in platelets. The 5-minute assay specifically detected *Staphylococcus* in platelets at concentrations as low as 1/16 of a McFarland 1 standard and is easy to interpret.

The assay is a rapid and sensitive test for detection of bacterial contamination in platelets and is feasible for routine blood bank use.

Improvements in sensitivity and addition of antibodies directed against other pathogens may allow extension of the outdated period of platelets to 7 days by providing a test to exclude contaminated units prior to transfusion.

Bacterial contamination of blood and blood products remains an uncommon but clinically devastating problem. Contamination most often stems from organisms introduced from the skin of the donor during venipuncture or rarely from donors with infection or asymptomatic bacteremia. Studies investigating the question have not clearly differentiated between contamination introduced by the study versus true contamination which can cause disease. A recent study confirming that bacteria-contaminated blood represents the most frequently reported cause of transfusion-related death to the FDA determined that the rate of transfusion-transmitted bacteremia in events/million units was 9.98 for single donor platelets, 10.64 for pooled platelets, and 0.21 for red cell concentrates. The study documented 34 cases and 9 deaths in a 2-year period, a rate exceeding the deaths caused by transfusion-associated viruses.

The only effective method for preventing transfusion-transmitted bacterial infections would be a pre-transfusion screening test of individual units. Numerous methods have been advanced to accomplish this including Gram staining, culturing, epifluorescence microscopy, bacterial ribosomal RNA gene probes, and multiplex PCR. Gram staining is relatively insensitive compared to the other methods. Culturing represents the gold standard but cannot be performed with instant results obtainable immediately prior to transfusion. We developed a rapid latex agglutination immunoassay for detecting bacteria in 5-day-old units of platelets. We chose *S. epidermidis* as an index bacteria for a proof of concept assay, since this organism is one of the most common contaminants of platelets.

**Materials and Methods**

Two strains of *S. epidermidis*, 12228 and 14990 (ATCC, Manassas, VA) were inoculated into rabbits by a commercial vendor (Animal Pharm, Healdsburg, CA) for production of rabbit anti-*S. epidermidis* polyclonal antiserum (anti-*Staphephi*-AS). The IgG fraction was subsequently purified (anti-*Staphephi*-IgG) by using ImmunoPure IgG (Protein A) Purification Kit (Pierce, Rockford, IL) according to the vendor’s instruction. Control IgG from the rabbit preimmune serum (Pi-IgG) was similarly prepared.

**Confirmation of Antibody Binding to Staphylococcus epidermidis**

A colony of *S. epidermidis* was taken from a cultured blood agar plate and then smeared on each albumin-coated glass slide (Snowcoat X-tra, Surgipath, Richmond, IL), followed by brief heat-fixation at 60°C. One drop of the purified anti-*Staphephi*-IgG, or anti-*Staphephi*-AS, was added to each bacterial smear. Preimmune-IgG and preimmune serum were added to bacterial smears as controls under the same conditions. The slides were incubated in a moisture chamber for 30 minutes at 37°C, then rinsed with phosphate buffered saline pH 7.2 (PBS), then with water. One drop of FITC-goat-anti-rabbit-IgG-antibody was diluted 1:160 in PBS (Research Diagnostics, Flanders, NJ) and added to each bacterial smear. The slides were again incubated and rinsed as above. Fluorescence was immediately observed using an epifluorescent microscope (Olympus Optical, Tokyo, Japan) equipped with a camera.

**Preparation of Latex-anti-Staphephi-IgG**

Polystyrene latex microspheres PS03N/4670 (Bangs Lab, Carmel, IN) were diluted with PBS to concentrations of 10 mg/mL per tube. The latex suspension was then mixed with various concentrations of *Staphephi*-IgG, incubated at room temperature for 3 hours and washed, and centrifuged 3 times using PBS. The microsphere pellet (latex-*Staphephi*-IgG) was then resuspended in PBS with 0.05% bovine serum albumin (BSA) at a concentration of 10 mg beads/mL. Latex-Pi-IgG was similarly prepared.

**Agglutination Test for Latex-anti-Staphephi-IgG**

To compare the detection intensity of latex-anti- *Staphephi*-IgG, 1 drop of beads bound with various concentrations of anti-*Staphephi*-IgG was added.
to a circle on Murex Biotech paper. Latex-Pi-IgG was used as a negative IgG control. One colony of S. epidermidis 12228 (or 14490) was then added to the circle and mixed with the beads. Five minutes later, the agglutination results were observed and compared to negative controls consisting of the beads without addition of the bacteria and arbitrarily scored using a 1+ to 4+ grading system. The concentration of anti-IgG demonstrating strongest agglutination was chosen for the following experiments.

The detection sensitivity of the latex-anti-Staphepi-IgG was determined by adding 1 drop of the chosen beads into each circle on paper and a drop of various concentrations of S. epidermidis. Five minutes later, the agglutination results were observed and scored as mentioned above.

### Specificity of Latex-anti-Staphepi-IgG

The specificity of latex-anti-Staphepi-IgG was determined by mixing 1 drop of latex-anti-Staphepi-IgG (1:5, v/v) with 1 colony of either S. epidermidis, S. aureus, S. saprophyticus, or S. pneumoniae within circles on the Murex Biotech paper. Five minutes later, the results were scored.

### Agglutination Test for Latex-anti-Staphepi-IgG

Latex-bound anti-Staphepi-IgG in the concentration (v/v, ie, volume of IgG/total volume of latex suspension) range of 1:5 to 1:20 detected S. epidermidis on test paper. Latex-anti-Staphepi-IgG (IgG 1:5) consistently showed the strongest agglutination for the detection and was chosen for other agglutination tests.

The latex-anti-Staphepi-IgG detects S. epidermidis in concentrations down to the equivalent turbidity of a 0.5 McFarland standard, in a concentration-dependent manner, as observed microscopically. There was marked agglutination when latex-anti-Staphepi-IgG mixed with S. epidermidis, but no agglutination with the other Staphylococcus species or with S. pneumoniae.

### Clinical Platelet Assay

Addition of the platelet suspensions to latex beads renders the latex nearly invisible on black or white paper. The latex-antibody detection test for platelets was therefore examined under the microscope using hanging-drop glass slides using the 4+ scoring criteria. Latex-anti-Staphepi-IgG (1:5) detected S. epidermidis in platelet product in a dose-dependent fashion. Panels 1 through 8 of Fig. 1 show 0 to >2.0 McFarland standards of S. epidermidis (original magnification x400).

We detected S. epidermidis contamination in platelets as low as the equivalent turbidity of a 1/16 dilution of a 1.0 McFarland standard or about 1 × 10^7 colony forming units per mL. The assay takes less than 5 minutes, giving an answer in real time with no need for sophisticated equipment. We chose S. epidermidis as the organism to study for proof of concept because of its ubiquity. We envision a clinical
assay would contain a mixture of latex particles, each conjugated with an antibody directed against a specific bacteria such as *S. viridans*, *Salmonella* species, *S. aureus*, *S. epidermis*, *Propionibacteria* species, *Enterobacteriaceae*, and *Pseudomonas* species. In addition, the conditions of the test, including antibody concentration and affinity, would be optimized to detect approximately $10^5$ colonies, a sensitivity now achievable with the more laborious and time-consuming acridine orange method. A sample of platelet concentrate would be removed from the available tubing link or directly from the unit using a sterile docking device, then assayed. Any positive agglutination would indicate the presence of 1 of these organisms and the need to quarantine or discard that unit of platelets.

Our current detection method employs microscopic observation of agglutination. Alternatively, the method could be adopted to use colored latex particles that could be visibly identified without the use of a microscope as a ring or pellet when centrifuged through a gel.\(^{15}\) When fully developed and tested in actual units of platelets destined for transfusion, either approach would provide an inexpensive, technically simple, and immediate response assay to detect bacterial contamination of platelets.