Comparison of the Bactec 9240 and BacT/Alert Blood Culture Systems for Evaluation of Placental Cord Blood for Transfusion in Neonates

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The Bactec 9240 and the BacT/Alert blood culture systems were compared as a means for detection of bacterial contaminants in whole blood, concentrated red cells, and plasma preparations prepared from umbilical cord blood (UCB) samples. Ninety-two UCB units seeded with low levels of various bacteria were evaluated. In more than 50% of cases, growth was not detected in plasma using either system (P < 0.001). When concentrated red cells and whole blood were compared, the Bactec system detected bacterial growth consistently sooner than the BacT/Alert system in all seeded bacteria except Staphylococcus species in whole blood. The median lengths of time to detection (LTD) for whole blood and concentrated cells in BacT/Alert were 18.7 h and 18.5 h, respectively. The median LTD for the same blood fractions using the Bactec system were 16.05 h and 15.64 h. These differences in LTD by blood culture system and sample type were statistically significant (whole blood, P = 0.0449; concentrated cells, P = 0.0037). Based on the results of our study, we recommend the use of either concentrated red cells or whole blood for sterility testing in UCB samples. In our laboratory, the Bactec system compared to the BacT/Alert system was the superior method for rapid detection of bacterial contaminants in cord blood.

While all neonates experience a decline in their circulating red blood cells immediately after birth, anemia is a more common complication for premature neonates (27, 28). Annually in the United States, an estimated 130,000 anemic, critically ill infants receive approximately one million red blood cell transfusions (31). Autologous blood transfusions have been shown to be safe in both adult and pediatric patients (17, 21, 25). Umbilical/placental cord blood is autologous blood from a neonate (20), and the use of autologous umbilical cord blood (UCB) has long been discussed among neonatologists (5, 6, 9, 10, 29). Owing to the increasing utilization of UCB for the transplantation of hematopoietic stem cells, significant progress has been made in developing safer and more efficient collection techniques for UCB (12, 14). In neonates, bacterial contamination has been described as the third most common cause of transfusion-related fatalities, with most fatalities occurring in gram-negative sepsis (13). Unfortunately, many cases of transfusion-transmitted bacterial infection remain unrecognized and underreported (4, 18, 30). While much experience exists now regarding the efficacy, recovery, and safety of UCB, only few studies investigated the prevalence of bacterial contamination of cord blood. These studies report variable bacterial contamination rates of between 1.85 and 12% (3, 7, 8, 10, 14). Bacterial contamination consists predominantly of organisms known as typical skin contaminants similar to those described in adult blood culture collections. Organisms of the vaginal flora have been described as an additional and important component of contaminants in UCB. The American Association of Blood Banks (AABB) standards require that a small volume of collected UCB be used for sterility testing. While general regulations exist for the evaluation of safety, including bacterial and viral pathogens, in adult blood and platelet collections as well as human cell therapy products, to our knowledge no specific method requirements for the evaluation of bacterial contamination of UCB for autologous transfusions have been published to date (1, 11). The two most frequently used FDA-approved automated, continuous-monitoring blood culture systems in the United States are the BacT/Alert system (bioMérieux, Durham, NC) and the Bactec system (BD Microbiology, Sparks, MD). In the current study, we investigated the performance of the Bactec 9240 and BacT/Alert continuous-monitoring blood culture systems for the detection of “seeded” bacterial contaminants in UCB samples compared to adult blood collections, with an additional focus on detection of “seeded” bacteria in various fractions of cord blood components.

MATERIALS AND METHODS

Between 2007 and 2008, 97 UCB samples and 61 adult blood samples were collected at the University of Iowa Hospitals and Clinics (Children’s Hospitals and the DeGowin Blood Center), Iowa City, IA. Additional cord blood collections from neonates were obtained at the Genesis Medical Center, Davenport, IA. The study protocol was approved by the Institutional Review Boards at all participating hospitals. The study was conducted in three phases, first evaluating the contamination rate for UCB collection, using a specific collection method, and second evaluating the performance of the Bactec and BacT/Alert continuous-monitoring blood culture systems for detection of organisms in seeded UCB samples. During the final, third phase, we evaluated both blood culture systems...
for detection of organisms in adult blood collections as a comparison to UCB samples. For placental cord blood collections, written informed consent was obtained from a parent prior to delivery of the infant. Eligible patients were those born to mothers older than 18 years with delivery between 23 and 41 weeks of gestation. Exclusion criteria were clinically suspected and/or laboratory-confirmed fetal anemia, major congenital malformations, or chorioamnionitis. Because a large number of neonates requiring transfusions of various blood products are preterm infants, equal numbers of preterm and term infants were enrolled in this study.

For adult patients older than 18 years, written informed consent was obtained prior to phlebotomy. Eligible patients were those with the diagnosis of hemochromatosis (prior confirmation by genetic analysis) who were undergoing therapeutic phlebotomy during maintenance phase of their disease. The maintenance phase was defined by normal iron status (ferritin, <50 g/dl, transferrin, <35%) and a greater-than-4-femtoliter decrease in mean erythrocyte corpuscular volume. Patients with diabetes mellitus and/or clinical or laboratory evidence of liver disease during the previous 2 years were excluded from the study.

Collection of UCB and adult blood. After delivery of the newborn and immediately after delivery of the placenta, the umbilical cord was cleansed using a povidone-iodine scrub followed by an isopropl alcohol swab and allowed to dry for 10 to 15 s before needle puncture. Using a gravity-based method system, placental/UCB was then collected into 250-ml blood collection bags (12). Each collection bag contained 33 ml of citrate phosphate dextrose (CPD) anticoagulant storage medium (Fenwal single blood-pack unit, Lake Zurich, IL; product code 4R0837MC). On average, 47 ml of cord blood was collected, accounting for total volume of 80 ml per collection bag. Procedures followed manufacturer’s and published guidelines for cord blood banking. Adult blood collections from phlebotomies for hemochromatosis maintenance-phase therapeutic interventions were collected into CPD-containing blood collection bags (63 ml CPD per bag), achieving a final volume of 420 to 450 ml of whole blood/CPD per bag. All procedures followed blood donation and collection guidelines of the AABB (2).

Study design for phases 1 to 3. During phase 1, 10 ml of cord blood was collected into a Wampole Isolator blood tube lysis centrifugation system (Wampole Laboratories, Cranbury, NJ) for 68 consecutive cord blood collections at the University of Iowa, Hospitals and Clinics. Isolator blood tubes were processed according to the manufacturer’s guidelines, and the samples were plated onto sheep blood agar, chocolate agar, and eosin methylene blue agar. All agar plates were examined for bacterial growth at 24, 48, and 72 h of incubation (35°C, 5% CO2 atmosphere).

During phase 2, 97 UBC collections were collected and subsequently inoculated with 97 recent clinical laboratory isolates of various bacteria (Table 1). The bacterial isolates were selected as being representative of those bacteria most frequently isolated from clinical cord blood samples (13, 14, 18). Suspensions of test organisms approximately equivalent to 102 CFU/ml were prepared in Trypticase soy broth. Using a sterile coupling device, an aliquot from the final stock solution was aseptically transferred into the blood collection bag, achieving a final organism concentration of less than 10 CFU/ml per blood collection bag. This target concentration was verified by culture quantitation of an aliquot from each final blood stock. Seeded cord blood preparations were gently agitated for approximately 5 min. Using a single 20-ml syringe, 16 ml of cord blood was aseptically withdrawn using a 21-gauge needle. Eight-milliliter aliquots of this sample were then immediately transferred aseptically into BacT/Alert FA, FAN Aerobic and Bactec Plus Aerobic/F bottles. The remainder of the seeded cord blood sample was then centrifuged (1,000 × g for 10 min) for separation of concentrated erythrocytes and plasma. The procedures followed AABB recommendations for processing blood and stem cell component donations. The plasma fraction (PF) was aseptically removed using a single 20-ml syringe, leaving the concentrated erythrocyte fraction in the original blood collection bag. Using aseptic technique, 8-ml aliquots of plasma were transferred into BacT/Alert FA, FAN Aerobic, and Bactec Plus Aerobic/F bottles. Finally, corresponding aliquots of concentrated erythrocytes were aseptically removed from the blood bags and transferred into the corresponding blood culture bottles. In some cases (n = 34), less than 16 ml (range, 2 to 14 ml) of concentrated erythrocytes had remained in the blood collection bag. In these cases, the remaining volume was equally split for inoculation into BacT/Alert FA, FAN Aerobic, and Bactec Plus Aerobic/F bottles (mean volume, 4 ml per blood collection bottle). All inoculated bottles were immediately placed on their respective continuous-monitoring instruments and incubated for a period of up to 5 days (120 h). For all samples and specimens, the order of bottle inoculation was random to ensure that each bottle was inoculated at approximately the same number of times.

The time that bottles first registered as being positive was recorded. Subcultures of positive bottles were performed to ensure that the organisms that grew were the same as the organisms used to seed the respective cord blood samples. The lengths of time (in hours) to detection (LTD) for each system for all tested organisms were compared.

During phase 3, a total of 10 adult blood samples were collected and divided into equal aliquots similar to the volumes of cord blood samples. This was done to ensure that a corresponding number of different bacterial organisms was inoculated, matching the corresponding UCB samples. Each adult blood collection bag contained 63 ml of CPD anticoagulant storage medium to account for an equal concentration of citrated compared to cord blood samples. As described above for UCB, each aliquot of adult blood was inoculated with a microbial suspension of the same selected organisms for UCB, achieving a final organism concentration of less than 10 CFU/ml per blood collection bag. After gentle agitation, 8 ml of adult blood was aseptically transferred into BacT/Alert FA, FAN Aerobic, and Bactec Plus Aerobic/F bottles and immediately placed on the corresponding continuous-monitoring system. Bottles were incubated for a period of up to 5 days. The LTD was determined as described above for UCB. Plasma and concentrated red cell components were not prepared and tested for adult blood samples.

Statistical analysis. Blood cultures were defined as negative after 120 h of incubation without growth, and with positive cultures, the LTD (in hours) was used for the analysis. Recovery of the organism (growth/no growth) was evaluated using the Fisher exact or chi-squared test. The LTD was analyzed using the Wilcoxon rank-sum (Mann-Whitney) test. Measures of association and descriptive statistics were performed using Stata 9 (Stata Corporation, TX).

RESULTS

During phase 1, 68 Isolator blood tubes were collected. Of these, 63 did not yield any growth of bacterial organisms after 72 h of incubation. Four of five samples with growth had coagulase-negative staphylococci (CoNS) at day 3 of inoculation, and one other sample was positive for CoNS at 48 h. All positive samples demonstrated growth of a single colony on a single type of medium only. We postulate that these organisms represent contaminants in the laboratory (e.g., from contamination at the time of collection) rather than being true pathogens present in the cord blood, since only one of three media (sheep blood agar, chocolate agar, and methylene blue agar) per sample demonstrated growth in each case after at least 48 h of incubation.
During phase 2, an analysis for bacterial growth in either blood culture system compared results for the three tested compartments (whole blood, concentrated erythrocytes, and plasma) in 92 of 97 cord blood samples. In five instances comparisons were not possible due to absence of at least one compartment being used for inoculation and testing. These five comparisons were not possible due to absence of at least one compartment being used for inoculation and testing. Five samples were excluded from further analysis. For the remaining 92 samples, bacterial growth was detected more often in whole blood and concentrated erythrocytes than in plasma (Table 1). This was statistically significant using Pearson’s chi-square test ($P$ value of <0.001 for comparison of whole blood versus plasma). Compared similarly to whole blood, bacterial growth was more often detected in concentrated erythrocytes ($P$ value of <0.05); however, this comparison was not as strong as the comparison to plasma.

Based on these findings, the results for plasma were excluded from further comparison of the Bactec and BacT/Alert systems for evaluation of mean LTD.

The difference in LTD in the two blood culture systems for individual organisms is presented in Table 2. Overall, a shorter LTD was observed for the Bactec system than for the BacT/Alert, 1.64 h. These differences, however, were not statistically significant ($P = 0.32$ for Bactec and $P = 0.68$ for BacT/Alert). Therefore, detailed data on comparison of adult and cord blood are not described further.

## DISCUSSION

The use of UCB as the means of autologous blood transfusions is a novel and emerging form of treatment for anemia in critically ill and preterm neonates. To date the use of cord blood is still subject to clinical investigations, and universal guidelines for harvesting, processing, and utilization have not been developed. Current guidelines within the 21 CFR 1271 in the Code of Federal Regulations (CFR) address sterility testing for cell therapy products. UCB is listed in this section; however, the regulations in 21 CFR 1271 do not explicitly require a specific method to be used for sterility testing in cell therapy products, including UCB (1, 11). The results of our study clearly indicate that either whole blood or concentrated erythrocytes should be the preferred specimen for detection of bacterial organisms in UCB. The plasma fraction appears not to be a suitable surrogate medium for sterility testing. These findings are contrary to those described by Honohan et al., who stated that cord blood did not show a preferential location of bacteria after centrifugation and prior to processing for transfusion (15). The higher centrifugation speed (1,000 g) used in our study compared to the study by Honohan et al. (50 g), as expected, resulted in organisms to be more likely to be concentrated within the red cell fraction rather than within the plasma fraction. Additional studies are necessary to further
evaluate the effects of centrifugation speed on the concentration of bacteria in various fractions of blood. The differences for organism recovery in the plasma fraction were most striking for CoNS, GBS, and \textit{S. aureus}. These organisms represent important UCB contaminants and also are significant causes of neonatal bacteremia (13, 24, 30). In further support of our findings, we identified other studies investigating the utility of UCB for autologous transfusions in neonates that have shown successful use of either whole blood or concentrated red cells for detection of bacterial contamination with adequate organism recovery (4, 9, 16). The majority of laboratories in the United States use either the BacT/Alert system (bioMérieux, Durham, NC) or the Bactec system (BD Microbiology, Sparks, MD) as their automated continuous-monitoring blood culture system. Khuu et al. found that the Bactec and BacT/Alert automated blood culture systems are at least equivalent if not superior to the CFR-based culture methods for detection of bacterial contaminants in UCB and other human cell therapy products (16). The results of our study indicate that when using the LTD as a basis of comparison from seeded samples, the Bactec system was statistically superior to the BacT/Alert system for detection of bacteria in UCB. These observations are consistent with the findings by other authors who compared the performances of the Bactec and BacT/Alert systems (23, 26). However, it is important to mention that both blood culture systems had a less-than-optimal recovery of GBS and \textit{S. aureus} in whole blood (20% and 50%, respectively), whereas recovery was much better for these bacteria in concentrated red cells (30% and 100%, respectively). The initial inoculum concentrations for seeded neonatal cord blood samples were rigorously verified by colony counts and had a mean of 1.7 CFU/10 μL (range, 0 to 7 CFU). We postulate that in the effort to seed cord blood samples with a low concentration of organisms, some bottles by chance may initially not have received a large enough viable inoculum size. This finding could be attributable to the overall small sample size for GBS and \textit{S. aureus} in this study. Furthermore, the presence of citrate from the CPD preservative used for cord blood banking in our study may have had a negative effect on the ability of certain organisms to grow. A low recovery rate for GBS and \textit{S. aureus} was also observed in a study by Smith et al. demonstrating the possible effects of different additives present in the blood culture bottles on bacterial growth (26). The bactericidal activity of citrate and other organic acids has been previously described by Lee et al. and Richards et al. in two independent studies (19, 22). Additional studies with larger sample sizes examining the effects of such additives are necessary to detect possible differences in overall recovery rates and LTD between different blood culture systems for these particular organisms in UCB. Although the results were not statistically significant, both blood culture systems detected growth faster in cord blood than in adult blood, and the Bactec system registered bacterial growth in adult blood consistently faster than the BacT/Alert system. The lack of statistical significance in observations for adult versus cord blood may be due to the small sample size and the use of a specific adult population (hemochromatosis patients) in this study. Additional studies comparing the use of different methods for detection of bacterial contaminants in adult and cord blood may be necessary to further evaluate the differences in LTD by system and blood component fractions. In summary, the most important conclusion from our work is that plasma appears to be a substandard specimen for the detection of bacterial contamination of UCB intended for transfusion in neonates. Either whole blood or concentrated red cells postcentrifugation should be the preferred specimen type. We believe that centers utilizing UCB for autologous transfusion in neonates may select and implement a continuously monitoring, automated blood culture system for sterility testing after appropriate on-site validation has been performed. In addition, we conclude that in our laboratory, the Bactec system is a better method than the BacT/Alert system for the screening of UCB units because of more rapid detection of bacteria.

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