

## Hematology

### WHOLE BLOOD COAGULATION TESTING IN NEONATES

A common hemostasis laboratory problem involves performing routine and specialized coagulation procedures on neonatal patients. Many advancements have been made in medical technology that use small (micro) samples of blood for multianalyte testing for neonates. Two common procedures, the prothrombin time (PT) and activated partial thromboplastin time (APTT), require a large amount of blood and have several factors that influence the testing variables. These factors include alterations in the blood-to-anticoagulant ratio and variations in the venipuncture procedure, in preparation of plasma, in reconstitution of reagents, and in use of instrumentation.<sup>1-5</sup>

The PT and APTT are important tests for monitoring the coagulation system in some neonates. The coagulation system, as with other systems in the premature infant, is not fully developed—which is reflected by the deficient amount of coagulation factors. This condition may predispose the infant to bleeding.<sup>6</sup> Volpes's recent studies suggest that the premature infant with an abnormal PT/APTT is at increased risk for intracranial hemorrhage and may benefit from coagulation factor replacement.<sup>7</sup> Other conditions may also predispose infants to increase consumption of coagulation factors (disseminated intravascular coagulation), including placental abruption, severe respiratory distress, asphyxia, and infection.<sup>8</sup> Thus, frequent monitoring of the integrity of the coagulation sys-

tem is important in these infants.

All laboratorians who have worked in the coagulation section of the laboratory have heard the question "What's the least amount of blood you need to perform a PT/APTT on a newborn? How much blood can you get away with to run these tests?" One of the major problems with the present laboratory techniques is that it usually requires 3.0 mL of sample (2.7 mL blood to 0.3 mL anticoagulant) to perform these tests. This amount of sample may represent 5% of the total blood volume of a premature infant. Serial testing will require transfusions for blood replacement, which carries added risk.<sup>8</sup> There are now methods of measuring these coagulation indicators using very small amounts of blood that can alleviate the long-time laboratory problem of "how much blood is enough . . ." and the variables that can influence plasma coagulation procedures.

At our facility we recently evaluated a coagulation system, the Biotrack 512 coagulation monitor, manufactured by Ciba-Corning Diagnostics Corp. (Medfield, Massachusetts). This device gave us PT/APTT methods that require only small volumes of blood.<sup>9,10</sup>

We simultaneously compared the Biotrack 512 coagulation monitor, which is a small hand-held device against a MLA 1000C automatic coagulation timer (Pleasantville, New York), on samples of blood obtained from infants in

the neonatal intensive care unit (NICU). Previous studies comparing the Biotrack 512 system on fingerstick specimens from adult subjects on anticoagulation therapy with other standard coagulation laboratory procedures showed good correlation.<sup>9-11</sup>

This study was accomplished by comparing nonanticoagulated whole blood from heel sticks, umbilical venous blood, and umbilical arterial blood against citrated anticoagulated blood samples. A total of 38 infants admitted to the NICU requiring a central intravenous (i.v.) line were included in this study. The central i.v. line was flushed with normal saline before being placed. After placement of the central line, 2.0 mL of blood and normal saline were removed to clear the line. A 2.7-mL sample of fresh, whole blood was drawn and dispensed in a siliconized, 3.8% sodium citrate blue-stoppered Vacutainer tube and sent to the laboratory for PT/APTT testing with the photo-optical MLA 1000C system with Baxter/Dade reagents (Miami, Florida). The PT reagent was Thromboplastin C and the APTT reagent was Actin with 0.02 M calcium chloride. Two 0.1-mL samples of whole blood in syringes were also drawn and used for PT/APTT testing with the Biotrack 512 coagulation monitor. This system uses a new type of laser optical system. The reagents are dry chemicals contained in individual disposable test cartridges for PT and APTT testing.<sup>9,10</sup> Only a single drop of blood is required (approximately 25  $\mu$ L for PT and 45  $\mu$ L for APTT) for each test. The drop of blood is applied directly to the sample application well of a PT or APTT test cartridge containing a reagent

chamber and a reaction path.<sup>9,10</sup> The blood is drawn by capillary action into the reagent chamber where it mixes with the reagents to initiate coagulation.<sup>9,10</sup> The blood and reagent mixture moves along the reaction path until a clot forms. The time from the application of the sample of blood to the cartridge to cessation of flow is detected by the laser optical system and converted to the PT/APTT plasma equivalent.<sup>9,10</sup> This device is a hand-held system that can be run on AC or DC power and is approximately 5  $\times$  9  $\times$  2 inches.<sup>11</sup> All testing with the monitor was performed at bedside. All of the blood collections for the MLA 1000C and bedside Biotrack 512 testing were performed by a single operator. The turn-around time for MLA testing was approximately 90 minutes. The Biotrack 512 usually gave results in approximately three to four minutes for both the PT and APTT testing.

The results of our study need to be viewed in the context of clinical relevancy. Here are the data:

Prothrombin Results	MLA 1000C	Biotrack 512
Number of samples	38	38
Mean (seconds)	13.4	14.7
Standard deviation	1.65	1.51
Correlation coefficient	0.81	
APTT Results	MLA 1000C	Biotrack 512
Number of samples	38	38
Mean (seconds)	45.3	54.7
Standard deviation	15.8	19.6
Correlation coefficient	0.81	

Clinically there is little difference between the means of the PTs and the APTTs. The neonatologists indicated they would look at the mean results as being essentially the same for clinical purposes in both testing coagulation systems described above.

Coagulation testing using this whole blood system has great potential. Previous studies have cited the validity of the Biotrack 512 system for monitoring patients on coumadin therapy.<sup>9-11</sup> This study evaluated the system for monitoring NICU patients. This system also has exciting potential for monitoring infants undergoing extracorporeal membrane oxygenation. Further use of this system in emergency rooms, surgical intensive care units, and operating rooms will soon be explored.

Whole blood coagulation testing will probably not be able to replace the classical coagulation testing, but it does provide some options for use in neonatal and emergency situations.

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## Immunology/Immunohematology

### RENAL TRANSPLANTATION ACROSS THE ABO BARRIER: THE MIDWEST ORGAN BANK EXPERIENCE

Solid organ transplantation has been governed by the conventional rules of blood group compatibility. Failure to heed the ABO blood group barrier generally results in hyperacute rejection of the organ. Such a rejection is mediated by the reaction of the recipient's anti-ABO isoagglutinins with the incompatible blood group antigen on the endothelial cells of the transplanted organ, as would occur if an A<sub>1</sub> or B kidney was transplanted into an end-stage renal disease (ESRD) patient who was blood group O.

In view of the weaker immunogenicity of the A<sub>2</sub> blood group antigen when compared with A<sub>1</sub>, in 1986 we began transplantation of A<sub>2</sub> or A<sub>2</sub>B kidneys into ESRD patients with blood group O or B,<sup>1-3</sup> after the initial report by Brynger et al.,<sup>4</sup> who successfully transplanted kidneys from A<sub>2</sub> donors into O patients.

#### Results

The procedure of screening all blood group A donors for the A<sub>2</sub> subgroup resulted in transplantation procedures in 33 patients with ABO-incompatible kidneys between 1986 and 1991, 24 who were blood group O and nine who were blood group B recipients. Of the 33 patients, 31 received cadaver-donor kidneys, and two received living-related kidneys. There were 3 A<sub>2</sub>B cadaveric donors in the series. Kidneys from these donors were transplanted into three blood group B recipients.

*Patient survival:* The overall patient survival was 96% at one year. One patient died of a cerebrovascular accident two months after transplant nephrectomy.

*Graft survival and follow-up:* The overall graft survival of the 33 transplants in this series is 67%, with a mean overall follow-up time of 36 months (range: 6 to 72 months).

The incidence of graft nonfunction, defined as either a graft that never functioned, or one that functioned for several days after transplantation but was then lost to rejection within one month after grafting, was 24.2% (8 of 33 grafts).

The incidence of early, or primary, nonfunction appeared higher in the initial experience than in our overall series of renal transplants. This prompted an analysis of the levels of antibody against the A<sub>1</sub> blood group antigen to determine whether there was a correlation between the antibody level and nonfunction of the kidney.

Table 1 illustrates these data. Pretransplant antibody data were not available on all patients from the early experience. Both immunoglobulin G (IgG) and immunoglobulin M (IgM) anti-A<sub>1</sub> antibody titers were analyzed. IgM titers did not predict graft function well.

It became apparent early in the patient series that patients with a titer greater than or equal to 1:8 of IgG antibody to the blood group A<sub>1</sub> antigen were at a substantially greater risk of early nonfunction and