A year of superlatives for blood banking in the US

TRALI, emerging pathogens, such as Chagas disease, and bacterial contamination testing were among the key topics discussed at the 2007 annual AABB annual meeting and exhibition (October 20-23, Anaheim, California). The conference attracted over 7,000 people – one of the highest attendances on record – and the exhibition was the largest ever with over 200 exhibitors, write Kerri Weinert and Carrie Cresenzi of Boston Biomedical Consultants Inc

Blood donations in 2007 increased only slightly in the US (by an estimated +1%), influenced by several stimulating and opposing factors.

The lack of good and current national and regional data continues to challenge the industry, according to Jim MacPherson, executive director of America's Blood Centers. There is increasing demand, which is offset to some degree by hospitals trying to use less blood per patient – so called blood conservation efforts, ie usually cutting blood use by use of intraoperative cell salvage and haemodilution).

The American Red Cross (ARC) saw a slight drop in donors in fiscal 2007, attributable to deferrals as well as other factors, such as the shrinking donor pool. Conversely, the use of platelets is solidly on the increase, due to more "widespread" use of aggressive cancer therapies, as more and more oncologists go into suburban and rural areas.

Automation for NAT-based testing

Discussions at AABB 2007 were focused on multiplex testing, as no new instruments for automation of NAT-based testing were being shown. Chiron displayed its Procleix TIGRIS instrument and promoted new system software, featuring enhanced operator functionality and ISBT28 barcode reading capability.

Chiron's partner, Gen-Probe, continues work on its post-marketing study for the Procleix Ultrio assay. The initial pivotal study for the Procleix Ultrio assay was not designed to demonstrate yield (defined as HBV-infected blood donations that are negative based on serology tests for HBsAg and anti-HBc), and so was not granted a screening claim for HBV.

To recap, the Procleix Ultrio assay received FDA approval for use on the Procleix eSAS; however, the assay was approved to screen donated blood, plasma, organs and tissue for HIV-1 and HCV in individual blood donations or in pools of up to 16 blood samples, while approval for HBV at this time applies only to the detection of HBV (not donor screening).

After screening approximately 260,000 samples by November 2007, Gen-Probe announced that it had found its first yield case and was awaiting confirmation from the FDA. Once the second case has been identified, the company plans to submit a supplemental BLA to the FDA and expects to receive the screening claim; the typical review time for a supplemental BLA is 4-6 months. The ARC has drafted a protocol for its own yield study, which it plans to initiate in early 2008.

Roche displayed its cobas Amplicor and cobas s 201 systems. In August 2007, Roche's cobas TaqScreen WNV assay and cobas s 201 system received FDA approval, with commercial launch in September 2007, the company's first US approval on the cobas s 201 system.

In October 2007, Roche announced that the Japanese Red Cross (JRC) awarded the company a contract to supply the cobas TaqScreen MPX test (HIV-1 M/O, HIV-2, HBV, HCV) to screen the entire Japanese blood supply (five million blood donations annually); the contract will be effective in 2008. The decision was made following JRC's evaluation of Roche cobas s 401 instrument and Chiron's Procleix TIGRIS. The cobas s 401 instrument and cobas TaqScreen MPX test will, in 2008, replace the Roche AmpliNAT tests that have been in routine use in the three JRC NAT testing centres since 1999.

Roche's core message to customers at AABB 2007 was that it is committed to blood screening with several new products in development, including a duplex HAV/B19 test to add to its triplex cobas TaqScreen MPX test for blood centres, next-generation resolution level multiplex test, and next-generation automation.

Automation for IAS-based testing

Adding to the already-approved anti-HBc and HBsAg assay, in July 2007, Abbott received FDA approval for its PRISM anti-HCV test; licensing of a full complement of PRISM assays is expected within two years, with HIV-1/2 and HTLV-I/II still under development. At AABB 2007, the company also presented data on a new third-generation HTLV-I/II test (in development) on the Architect instrument for use outside the US where the Architect instrument is used for donor screening purposes. The assay uses recombinant antigens. 200 tests per hour can be processed on the Architect immunoassay system.

Compared to last year, Ortho-Clinical Diagnostics' focus at AABB was mainly on its BGT products rather than its next-generation Paradigm instrument, which was shown as a prototype at AABB 2005 and was promoted via in-depth video presentations at AABB 2006; development of the Paradigm had been halted in 2007. Within IAS-based testing, main promotional efforts focused on the Ortho *T cruzi* ELISA test system, which was FDA-approved in December 2006. OCD will seek FDA approval to expand use of its test for Chagas disease in tissue and cell transplants (cadaveric testing) and for general diagnostic purposes.

In an announcement that will affect the entire blood screening area, OCD announced in July 2007 that it will no longer distribute a complete set of FDA-recommended blood donor screening assays resulting from the decision by bioMérieux to phase out production of its microplate immunoassays, including the HTLV-I/II test used on the Ortho Summit System. Its HTLV-I/II kits will remain available until June 2008; the date is based on OCD's product volume. In order to comply with FDA requirements for HTLV testing, customers must implement an Abbott platform for HTLV-I/II or send testing to another FDA-licensed laboratory.

Abbott indicated that it plans to discontinue the provision of bead assays for donor screening and does not plan to install the bead system for new customers; it appears in the

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future that the only option available for the performance of screening for HTLV will be the Abbott PRISM. This affects the algorithm for donor re-entry recommended by AABB in a 1999 bulletin requiring the performance of two different screening assays to increase assay specificity.

Emerging analytes

Chagas disease continued to be a major topic at the AABB conference with updates on testing given by the ARC (during a scheduled presentation) and Blood Systems Laboratories (among other sites), which implemented routine donor screening for Chagas disease using OCD's test on January 29 2007; as a result, 75-90% of collected blood in the US is tested for Chagas disease.

To date, six indigenous cases have been found (and remain under investigation) – in Texas (three), California, Louisiana and Tennessee. Seven transfusion cases have occurred, including two in Canada; and three transplant clusters have also been found.

Within the ARC Prevalence Study, there were 32 confirmed positive cases at the three sites (Los Angeles; Tucson, Arizona; and northern California) with 239 Radioimmune Precipitation Assay (RiPA) positive since screening began, which leads to a 0.013% repeat reactive rate.

California and Florida have the highest confirmed positives. while Florida, Arkansas, and California have the highest frequency of positives. Experts in this area have stated that look-back studies from donors identified in the past year are either negative or incomplete so the ferment over the clinical impact of this test continues.

Babesiosis and malaria were also discussed at AABB 2007. Babesia, the agent that causes babesiosis, is transmitted by ixodes ticks, infects red blood cells and causes a flu/malaria-like illness that usually resolves. There is a lifetime deferral for history of babesiosis and currently no test is available. *Babesia microti* has caused 70 known transfusion cases worldwide since 1979.

For malaria, there is a 1-3-year deferral for risk, and currently no test is available; difficulties have been encountered when using PCR to test for the presence of parasites because of intermittent or extremely low level parasitemia.

Plasmodium, the agent that causes malaria, infects red blood cells and liver cells and is transmitted by mosquitoes; less than five transfusion cases have occurred since 1998. As a result, malaria has become more of a blood availability issue versus a blood safety issue, with additional questions to the donor questionnaire reducing specificity and positive predictive value. Based on seven years of ARC data, 1-2% of donors (up to 100,000) donors are lost based on travel-related deferrals.

Dengue virus

Dengue virus was another emerging pathogen that may become important in transfusion medicine that was discussed at AABB 2007 and highlighted in an abstract by Gen-Probe. It is a single-stranded RNA flavivirus that has four different serotypes that are all capable of infection. An infected individual has lifelong immunity for the one subtype if infected. Dengue virus is highly endemic in Asia and expanding in the Americas.

Gen-Probe has developed a TMA-based assay on the Procleix eSAS and Procleix TIGRIS that has demonstrated a sensitivity of 14 copies/mL and a specificity of 99.9% with all four subtypes detected. The ARC is performing two studies on infectivity and transmissibility, but it is still in the research phase.

Seven-day platelets

Bacterial contamination testing was emphasised at AABB 2007, with focus on seven-day platelets and point-of-issue testing. The Post Approval Surveillance Study of Platelet Outcomes, Release Tested (PASSPORT) is ongoing. PASSPORT is sponsored by Fenwal (formerly part of Baxter) and Gambro; the FDA required these companies to perform the post-market study for their collection devices and bags in order to obtain seven-day claims. To recap, PASSPORT involves testing expired platelet products from 50,000 platelet collections to assess the performance of the bioMérieux BacT/ALERT 3D system as a release test. Of note, the BacT/ALERT 3D system was only FDA cleared as a quality control (QC) test upon bioMérieux's data submission; it is only referred to as a release test in the Gambro/Fenwal PASSPORT protocol as bioMérieux has not received FDA approval for its system as a release test. The BacT/ALERT 3D release test is the only FDA-cleared bacterial detection method that permits seven-day storage of single donor platelet products under the approved PASSPORT study protocol.

Although the French company's QC test package insert indicates that use of two bottles is "strongly recommended," the FDA requires both aerobic and anaerobic bottles to be used for the release of seven-day platelets as part of the PASSPORT protocol. Of note, at AABB 2007, the company promoted its next-generation data management system, OBSERVA, non-interfaced version 3. The Windows XP platform OBSERVA software consolidates data and results from the BacT/ALERT 3D system; this system upgrade will be available to all blood centres in 2008.

As of October 2007, 29 organisations (47 sites) were participating in the PASSPORT study. By the end of Q2 2007, some 193,078 release tests had been conducted and 2,571 surveillance tests (expired platelet products) had been conducted; there have been five transfusion-related reactions and no deaths. These data were presented at an ABC-sponsored North American Platelet conference held on November 29-30 2007. Accrual in the PASSPORT study is ongoing, but slower than expected (less than a quarter of the data has been collected) due to blood centres not joining as quickly as thought (ARC is not participating) and the fact that the study was predicated by capturing products that were out of date, and, to date, fewer products have entered surveillance; seven-day dating reduces outdates so much that the number of units available for enrolment is very small.

As November 2008 approaches (see TRALI below), US blood centres are assessing ways in which they can comply with AABB guidelines for reducing the risk of TRALI from transfusion of platelet products stored in plasma. Many of these actions. such as Human Leukocyte Antigen (HLA)/ Human Neutrophil Antigen (HNA) testing of female donors, are expected to result in an estimated reduction in the size of the apheresis donor base. Participation in the PASSPORT study can help maintain the apheresis supply despite the expected reduction in apheresis donors due to TRALI limitations; currently 80% of the US platelet market consists of apheresis platelets.

Pall displayed its Acrodose PL System and new Acrodose PLus System. Pall had received FDA clearance of the Acrodose PL System two years ago, and it was initially applicable to blood centres in the US that used a Pall collection and filtration system for whole blood platelet collection. The Acrodose PL System produced a new platelet product that provides many of the benefits of apheresis platelets, but at a lower cost. It was the first whole blood derived system for pre-storage pooling (four to six platelet units) and culture-based bacterial testing of leuko-reduced whole blood derived platelets, resulting in a transfusion-ready product for hospitals. The Acrodose PLus System allows the pooling device to be used with any collection system providing blood centres increased flexibility with their currently established practices.

The Acrodose PLus allows for the pooling for up to six individual platelet units and then a sample is taken with the

Pall eBDS prior to moving from the pooled bag through a leukocyte filtration device to the final five-day storage bag; any blood collection system can be used versus just Pall's. The indications for the Pall eBDS have also been expanded to now allow for detection of bacteria in the prefiltered, non-leuko-reduced pool, providing the maximum shelf life of the final platelet (if leuko-reduction occurs prior to bacteria testing, the product must be held an additional 24 hours since the leuko-reduction filter removes bacteria as well as white blood cells, and any remaining bacteria passing through the filter must re-grow for 24 hours prior to sampling).

Use of the Pall Leukotrap RC System with RC2D Filter, provides flexibility when used with the Acrodose PLus System since the whole blood may be spun heavily for maximum plasma recovery and red blood cells, or using a soft spin for plasma, platelets, and red cells (the platelets may then be pooled with the Acrodose PLus System).

Both Acrodose systems provide a lower cost alternative to apheresis platelets and also allow for more effective use of whole blood derived platelet products. In addition, because the pooling of whole blood derived platelets dilutes the product, use of both Acrodose systems adheres to the AABB guidelines for TRALI management of avoiding use of high plasma volume blood products, especially those gathered from women who have been pregnant. Pall is developing a next-generation product that could be used for point-of-issue (POI) testing.

Rapid point-of-issue (POI) tests

Rapid POI tests received increasing attention at AABB 2007 with awareness efforts by Verax, GenPrime, and Immunetics. All three manufacturers' tests require some pre-processing and hands-on time, whether it is centrifugation, resuspension and mixing with reagents, for instance.

Verax occupied its own booth at AABB 2007 and its Platelet PGD test was also promoted in the Abbott booth (given the October 2006 distribution agreement). In September 2007, the Verax Platelet PGD received FDA approval as a rapid, qualitative immunoassay for the detection of aerobic and anaerobic gram-positive and gram-negative bacteria in leukocyte*reduced apheresis platelets (LRAP) as an adjunct quality control test following testing with a bacterial detection device cleared by the FDA for quality control testing of LRAP.

After receiving this approval, the company is going to work with the FDA on a whole blood derived platelet claim; data are expected to be submitted by year-end 2007/early 2008, with approval of the 510(k) in 2008. The Platelet PGD requires a centrifugation step with re-suspension of the bacterial pellet prior to applying a 500 µL sample to the cartridge; it has been shown to have a sensitivity of 103 to 105 CFU/mL sensitivity.

In October 2007, the AABB bacterial contamination standard task force sent a letter to Verax noting a missed opportunity for the use of the newly-approved Platelet PGD test. While acknowledging that the device will help improve platelet transfusion safety, the task force expressed concerns that it was not cleared for use in testing pools of platelets derived from whole blood at or shortly before the product is issued, which is "the most urgent unfilled need for detection of bacterial contamination".

The task force urged Verax to develop clinical trials to have the device approved for this use on non-leukocyte-reduced and leukocyte-reduced pools of platelets from whole blood. The concern is not that the FDA did not clear the product for whole blood derived platelets since that was not the Verax submission. Rather, experts are concerned that the regulatory pathway to that clearance is more complicated and stringent than is medically appropriate.

GenPrime announced in October 2007 that it had initiated multi-site clinical trials of its bacterial contamination test for platelets. The test is a rapid QC device for detecting the presence of aerobic and anaerobic bacteria found in contaminated platelets. The clinical trial will generate data for filing a 510(k). Based on a lateral flow technology platform, the test detects all of the pathogenic organisms known to contaminate platelets. In addition, GenPrime has developed an automated test reader and expects to complete the clinical trial and file the 510(k) in mid 2008.

Immunetics' BacTx (under development) is a rapid POI test for platelet bacterial contamination testing that is based on the detection of peptidoglycans (a universal component of the bacterial cell wall for both gram-positive and gram-negative organisms) using a peptidoglycan binding protein. This contrasts with GenPrime's and Verax's products, which utilise antibodies to specific antigens for the bacteria. The BacTx can be used for apheresis and pooled whole blood derived platelet units as well as whole blood platelet concentrates as individual units before pooling. It detects gram-negative and gram-positive bacteria (both aerobic and anaerobic bacteria) and utilises a 1 mL sample volume. Results are provided in less than one hour versus more than eight hours on the BacT/ALERT (detection in under 20 minutes for many bacteria, with 100% detection within 30 minutes at 104 CFU/mL). The sensitivity of the test is 103 to 104 CFU/mL and the company is developing an automated reader.

Molecular blood grouping and typing

Focus on DNA-based BGT methods (molecular BGT) for Rh, ABO determination, and extended genotyping increased at AABB 2007 compared to the prior year with more abstracts published using commercial and laboratory-developed methods. Molecular BGT may be used for antigen typing, determination of zygosity (particularly RHD), resolve discrepancies with serological testing, distinguish alloantibodies from autoantibodies, detect weakly-expressed antigens when a patient is unlikely to make antibodies to transfused antigen positive red blood cells, and to identify the molecular basis of unusual genotype results (especially Rh variant). Compared to traditional serological BGT methods, molecular BGT has the following advantages:

- variety of sample types may be used including non-leuko-reduced whole blood, buccal swab, dried blood spot, tissue, or amniotic samples (not limited to red blood cells);
- does not depend on antisera, which may not be available; and
- especially beneficial for multiple transfused patients.

However, there are no FDA-licensed molecular BGT products, which raises CLIA concerns, and the methods are very manual and technical (forcing centralisation of testing) and costly. In addition, data management solutions are needed to link the genetic information to the donor and automated, front-end sample extraction methods will be desired to bring this technology to the mainstream.

There are several different techniques that may be used for molecular BGT, including allele-specific PCR, PCR-RFLP, sequencing, and high-throughput testing. High-throughput testing was discussed in detail given its greater applicability to the blood donor screening area. Automated DNA extraction may utilise robotic instrumentation to process 96 samples in two hours. Microchip technology has been employed to detect multiplex PCR with numerous targets; the microchips may utilise oligonucleotides on glass slides or beads. BioArray Solutions and Progenika are two companies that offer molecular BGT.

The BioArray BeadChip utilises colour-coded beads selected and coated with DNA. The beads, which are 2 µm to 5 µm in diameter, are assembled and immobilised on a wafer chip. The Human Erythrocyte Antigen (HEA) BeadChip tests for 19 polymorphisms from within 11 blood group systems; HLA, RHCE, and RHD chips are also in development. Total processing time from DNA extraction to final results requires eight hours. Excluding DNA extraction, 96 samples may

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be processed in under five hours with less than two hours of hands-on time allowing for over 500 samples to be tested per shift with standard laboratory automation.

BLOODchip, which is expected to receive regulatory approval in Europe for use as an in vitro diagnostic in early 2008, was developed by the Bloodgen consortium with Progenika Biopharma SA of Spain as the commercial partner. BLOODchip is a microarray-based test that determines 116 SNPs in nine blood group systems (ABO, RhD/RhCE, MNS, Kell, Kidd, Duffy, Diego, Dombrock, and Colton). BLOODchip identifies many mutations that cause D-negative phenotypes and differentiates between RhD+, D-negative, weak D, and partial D alleles. In addition, it includes software that scores both hybrid and point-mutated RHD genes causing variant Rh expression.

Clearly, this technology will not be mainstream anytime soon, and will co-exist with current serology testing even when implemented, but nonetheless will enter the IVD testing realm over time. In the future, molecular BGT potentially offers a comprehensive automatable solution to what was a labour intensive testing process, but at a significant cost increase over current serological methodology.

TRALI

Compared to 2006, transfusion-related acute lung injury (TRALI) continued to receive significant attention at AABB 2007. As a result of the issuance of recommendations provided to help in reducing the incidence of TRALI, HLA testing companies exhibited at AABB 2007.

Full implementation of the measures relating to plasma components and whole blood were expected to occur by November 2007 and measures relating to platelet components as soon as possible, but no later than November 2008. In September 2007, AABB conducted an electronic survey to track progress in implementation of the recommendations; eligible participants were AABB institutional members that were either blood centres or hospital blood banks with over 1,000 units collected annually. For plasma, 86% of respondents indicated partial or full implementation as of September 2007 and the remainder indicated their intent to implement. 70% of responding blood centers had not yet begun implementation of TRALI risk reduction strategies for platelets. Approximately 90% of blood centres indicated that they planned to include HLA antibody testing of at least some plateletpheresis donors as part of their strategy.

Regarding the specific donor population to consider for testing, the majority of respondents indicated that they would base testing on pregnancy history, history of transfusion, or test all female donors. Blood testing sites are all thinking about testing strategies, but most have made no decision. The testing will be based on the above-listed factors if it is implemented, and because of donor loss, it is implementation that is controversial (not so much who to test if testing is performed). Many sites believe that the best approach may be to generally reduce recruitment and retention of female donors and increase that of male donors and let the donor base evolve, or some combination of both with very targeted testing.

As mentioned above, these recommendations attracted HLA testing companies, including One Lambda, Tepnel Lifecodes, and GTI Diagnostics, to exhibit at AABB 2007 in order to promote their products, anticipating that HLA (and HNA) testing might be adopted for TRALI management. One Lambda displayed the LABXpress, an automated Luminex-based HLA and HNA testing system, which is OEM supplied from Stratec. The system can perform HLA and HNA testing (HLA class I, including MICA; class II; and HNA 1a, 1b, 1c, 2, 4) and can process up to 100 analytes per test or up to 800 samples in eight hours. The assays, branded LABScreen, are available in mixed format for screening and single antigen for supplementary screenings. The LABScreen Single Antigen test allows you to identify both HLA class I and class II donor-specific antigens. Use of the LABScreen Singles test further allows you to create an antigen panel that emulates the antigen profile of the donor or donors.

Tepnel Lifecodes displayed the Janus instrument from Perkin Elmer to help automate high-throughput LifeScreen testing. This system provides testing from 400-600 antibody screens in an eight-hour shift. In addition to performing pipetting steps, similar to the One Lambda platform, it utilises the Luminex platform for detection and analysis. The LifeScreen Deluxe tests contains 7 class I beads and 6 class II beads, while the Lifecodes Single Antigen (LSA) is available in an LSA class I panel and an LSA class II panel.

GTI Diagnostics promoted the DonorScreen-HLA (combined class I and class II HLA screening test) on the QuickSTEP Automated ELISA platform, which provides full automation for its microtitre plate assays. The system can screen up to 176 donor samples (using four microtitre plates) with minutes of hands-on time. The system and the assays were submitted to the FDA shortly before the AABB conference.

Amidst the technologies available for HLA antibody screening to reduce risk for TRALI are complement dependent cytotoxicity, ELISA, flow cytometry and Luminex; the Luminex platform was deemed to have high sensitivity and the greatest availability with commercial kits from multiple vendors. However, no test is licensed for screening applications and the cost of testing is currently high with automation expected to equal NAT.

US biovigilance network

One topic of note at this year's meeting was a progress report on the US biovigilance network, also referred to as the USBVN, which is a public-private initiative guided by the Interorganizational Biovigilance Task Force (see funding below). Currently, plans call for the network to utilise voluntary reporting of data collection through a web-based system. Given the keen interest in moving the network forward, first there is an overarching need to reassure participants concerning mechanisms to prevent the accidental "disclosure of confidential information", a point well addressed by Dr James AuBuchon (see below) of Dartmouth-Hitchcock Medical Center and the chairman of the USBVN Working Group (for transfusion recipients).

The vision of the USBVN is: to design and implement a comprehensive biovigilance system in the US that will improve the outcomes of collection and transfusion and/or transplantation of blood components and derivatives, cells, tissues and organs.

In summary, among the many benefits of a vigilance network in the US are the following:

- more immediate corrective actions should lead to improved quality and safety;
- it has a sentinel function to detect emerging threats and otherwise "rare events" that may become apparent (when data are consolidated across multiple sources);
- the pooling of information across multiple institutions makes for a more powerful and thorough comparison (as standardised data are to be collected) than through individual site analysis; and
- provision of analysis and charting tools allowing a participating institution to track and trend its activities and compare these to national benchmarks.

If the USBVN meets its targets in 2008, during the next annual AABB national meeting the organisers hope to demonstrate some of the more developed elements of the system, including recipient haemovigilance, and to provide initial instructional training.

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