Canadian Blood Services
Société canadienne du sang

DIAGNOSTIC SERVICES ALBERTA/NWT

A YEAR IN REVIEW
January – December 2013

CANADIAN BLOOD SERVICES ALBERTA/NWT
DIAGNOSTIC SERVICES

SENIOR STAFF AND CONTACT INFORMATION

MEDICAL DIRECTOR
DR. JUDITH HANNON MD, FRCPC **780.431.8714**
judy.hannon@blood.ca

MEDICAL CONSULTANT
Dr. Gwen Clarke MD, FRCPC **780.735.4384**
gwen.clarke@albertahealthservices.ca

DIAGNOSTIC SERVICES MANAGER
Leanne To, BSc, MLT **780.431.8727**
leanne.to@blood.ca

PERINATAL LABORATORY	**TELEPHONE**	**780.431.8759** **1.866.258.8889**
	FAX	**780.431.8747**
CROSSMATCH/REFERENCE LABORATORY	**TELEPHONE**	**780.431.8765**
	FAX	**780.431.8779**

WEBSITE **www.blood.ca/diagnosticservices**

TABLE OF CONTENTS **PAGE**

TABLES AND FIGURES

A. Perinatal Laboratory

The Perinatal Laboratory within Diagnostic Services at Canadian Blood Services provides screening of pregnant women for blood type and red blood cell antibodies under a program funded by Alberta Health and Wellness. This screening provides information to assist physicians, midwives and nurse practitioners in ensuring the appropriate management of a pregnancy for both the mother and baby. Testing is provided for Alberta, Northwest Territories, Saskatchewan (Lloydminster area) and western Nunavut.

Testing Performed

Canadian Blood Services (Perinatal Laboratory) routinely performs the following tests:

- ABO/Rh blood type
- Screen for red blood cell antibodies
- Antibody identification, if antibodies are detected
- Antibody titre, if a clinically significant antibody is identified (unless Kell system antibody or a critical titre has previously been reported).

Specimen Type/Frequency

Mothers – Initial Testing – all women should be tested upon their first prenatal visit

Mothers – 26-28 Weeks Gestation - all Rh negative women should be retested at 26-28 weeks gestation. Rh positive women should also be retested at 26-28 weeks gestation when there is only one blood group result available (usually first pregnancy) or if the patient is at increased risk of allo-immunization (eg. previous transfusion, fetal trauma or procedure, IV drug use).

Mothers - Antibody Present - if an antibody is present which is known to cause hemolytic disease of the fetus/newborn (HDFN), it is recommended that specimens be submitted every four weeks until the third trimester. More frequent testing may be requested if the antibody titre rises rapidly or if clinical monitoring mandates that additional sampling would provide helpful information. Beginning in the third trimester, specimens should be submitted every two weeks until delivery.

Mothers - Postnatal - following delivery, specimens from the mother and her baby should be tested if the woman is Rh negative, has a clinically significant antibody or if the baby shows signs of hemolytic disease of the fetus/newborn (i.e. anemia or jaundice). Midwives or hospitals that do not perform transfusion medicine testing should submit samples to their referral hospital or to Canadian Blood Services. A fetal bleed screen is performed on the maternal sample when a Rh negative woman delivers a Rh positive (or weak D positive) baby. If the test is positive, a Kleihauer-Betke test is performed to quantitate the bleed (this test may also be performed if a specimen is too old to test by the routine rosette method used for fetal bleed screening).

Newborns (Cords) – cord blood or neonatal specimens may be submitted with the mother's specimen as noted above. Cord specimens that are not submitted for testing should be retained for 7 days in case the baby shows delayed signs of HDFN. ABO/Rh and direct antiglobulin testing is performed on cord or neonatal specimens submitted to Canadian Blood Services.

Fathers - when a woman has an antibody capable of causing HDFN, specimens from the father will be requested for antigen typing. This may assist in assessing the probability of the baby being affected by the antibody. Fathers' specimens may also be tested to assess Rh Immune Globulin (RhIG) eligibility of Rh negative mothers.

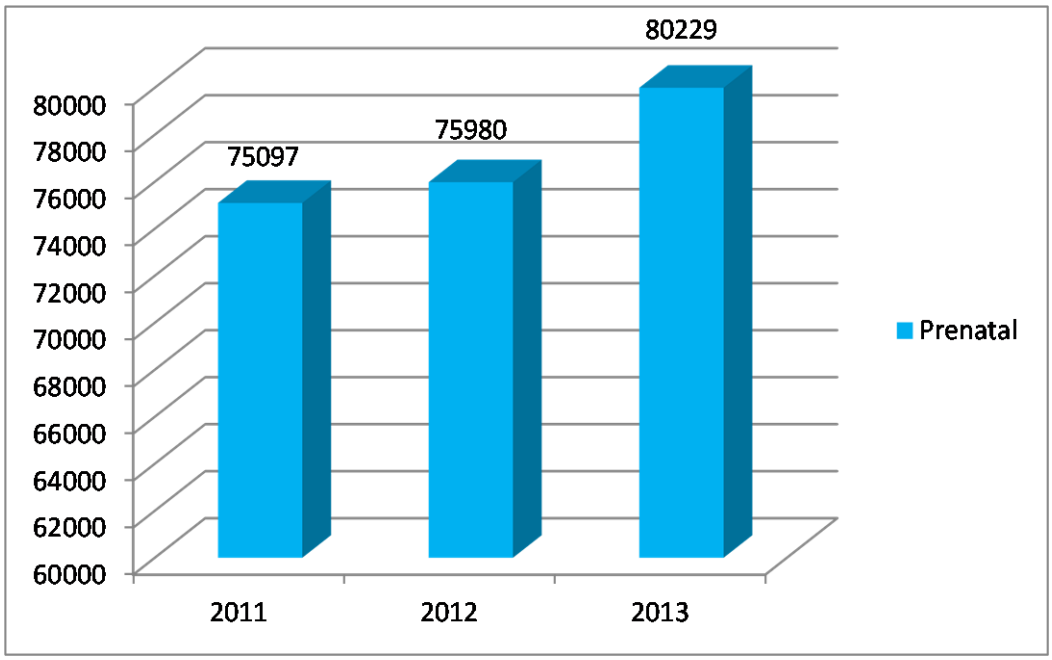
Specimens Tested

The total number of specimens tested rose by almost 6% from 2012 as seen in ***Table 1*** and ***Figure 1*** below.

Table 1: Perinatal Specimen Testing Totals

Specimen Source	*Test Type*	*2011*	*2012*	*2013*
Mothers	ABO/Rh, Antibody Screen	74641	75512	79718
Fathers	ABO/Rh	278	305	304
Cords	ABO/Rh, DAT	178	163	206
TOTAL		**75,097**	**75,980**	**80,229**
Patients Tested		**N/Av**	**63,277**	**68,877**

Figure 1: Total Perinatal Specimens Tested



Antibodies Identified

In 2013, a total of 557 antibodies were reported (***Table 2***). This is significantly higher than 2012. It is difficult to know what would account for this increase as there has been no change in our method of antibody detection or identification. However, a higher number of the 470 women with antibodies had more than one antibody (61 had 2 antibodies and 13 had 3 antibodies). Also interesting to note is that anti-Wr^a, a naturally occurring antibody to a low prevalence antigen that very rarely causes HDFN, was reported in combination with other antibodies in 10 of 11 cases so was likely an incidental discovery when investigating other antibodies.

Antibodies identified were considered to be clinically significant if they have been reported to cause Hemolytic Disease of the Fetus/Newborn (HDFN). The most common clinically significant antibodies identified were: anti-E, anti-c, anti-K and anti-D (***Figure 2***) which together represent over 60% of the total antibodies identified.

Table 2: Perinatal Antibodies Identified

Antibody	*Number Detected in 2011*	*Number Detected in 2012*	*Number Detected in 2013*
Anti-D	52	58	65
Anti-C	21	24	30
Anti-E	89	100	135
Anti-c	43	53	70
Anti-e	10	13	14
Anti-G	0	3	3
Anti-M	34	27	40
Anti-N	1	1	0
Anti-S	13	14	17
Anti-s	2	0	1
Anti-P1	1	0	0
Anti-Le^a	10	7	15
Anti-Le^b	3	2	6
Anti-K	43	49	70
Anti-Kp^a	0	0	1
Anti-Fy^a	11	11	11
Anti-Fyb	2	1	1
Anti-Jk^a	28	26	42
Anti-Jk^b	7	5	10
Anti-A$_1$	9	6	3
Anti-C^w	2	1	3
Anti-Di^a	1	2	0
Anti-Lu^a	0	0	3
Anti-Lu^b	2	2	4
Anti-V	1	0	1
Anti-V^w	0	0	1
Anti-Wr^a	0	0	11
Anti-Yt^a	2	1	0
Total	***387***	***406***	***557***

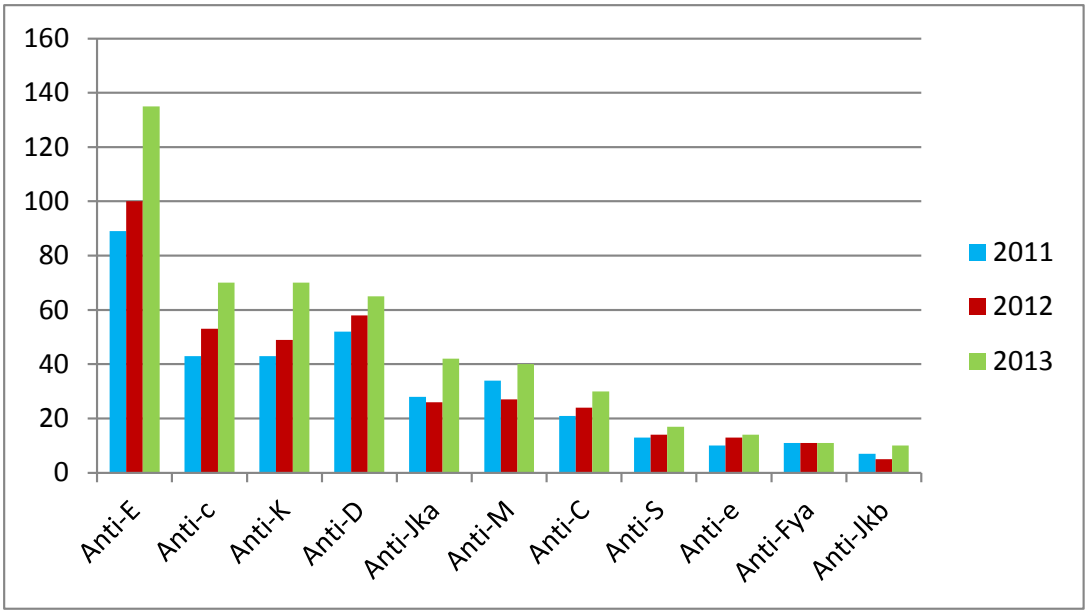
Table 3: Patient Antibody Titres

Antibody	*Critical Level*	*Non-Critical Level*	*Non-critical to Critical*
Anti-D	14	45	3
Anti-C	1	15	0
Anti-E	10	99	6
Anti-c	7	43	6
Anti-e	0	11	0
Anti-Fy^a	1	4	0
Anti-Fy^b	0	0	0
Anti-G	0	1	0
Anti-Jk^a	0	34	0
Anti-Jk^b	0	7	0
Anti-K	0	1*	0
Anti-M	0	27	0
Anti-S	2	11	2
Total	**35**	**297**	**17**

*Titres are not normally performed on Kell system antibodies as these antibodies may be critical at any level. This titre was performed upon request of the physician.

Titres for 17 of the clinically significant antibodies increased from non-critical to critical levels (≥16) during the pregnancy with a total of 35 antibody titres at critical levels (***Table 3***). Recommendations were made for all patients with a critical titre level (current or previous pregnancy) and all Kell system antibodies to be referred to a Maternal-Fetal Medicine clinic for further follow-up and monitoring during their pregnancy.

Figure 2: Relative Frequency of Clinically Significant Antibodies



Fetal Genotyping

Canadian Blood Services in Alberta has been referring out specimens for fetal genotyping (by amniocyte DNA testing) to the Blood Centre of Wisconsin for several years. This year we also began to refer specimens to the NHS in Bristol, England, as they can detect fetal DNA in maternal plasma.

Specimens are submitted through the Maternal Fetal Medicine clinics in Edmonton or Calgary and are accepted if they meet the following criteria:

- The mother has an antibody capable of causing hemolytic disease of the fetus/newborn (HDFN), AND
- The father is heterozygous for the corresponding antigen (or unknown), AND
- The antibody titre has reached a critical level, OR
- There has been a previous fetus/newborn affected by HDFN, OR
- The antibody is anti-K, OR
- Amniocentesis is being performed for another indication (i.e. advanced maternal age), even if the mother's antibody titre is at a non-critical level.

Discussion with a Canadian Blood Services physician is required prior to submitting specimens for testing outside of these criteria.

The number of specimens referred for fetal genotyping has ranged between two and eight specimens in recent years. In 2013, 5 amniotic fluid samples and 18 maternal blood specimens were tested from a total of 20 patients (a second blood specimen needed to be submitted for 3 patients). In 7 of the cases, the fetus had not inherited the gene(s) for the antigen against which

the maternal antibody was directed. These patients no longer required follow-up at a Maternal-Fetal Medicine clinic during this pregnancy.

Table 4: Fetal Genotyping Results Summary

Patient	Maternal Antibody	Source of Fetal DNA	Predicted Phenotype based on Fetal Genotype	Follow-up Required?
1	Anti-D	Amniotic Fluid	D+	Yes
2	Anti-E	Amniotic Fluid	E+	Yes
3	Anti-D, Anti-C	Maternal Plasma	D+	Yes
4	Anti-K	Maternal Plasma	K-	No
5	Anti-D	Maternal Plasma	Unable to type	Yes
6	Anti-K	Maternal Plasma	K-	No
7	Anti-D	Maternal Plasma	D+	Yes
8	Anti-E	Amniotic Fluid	E+	Yes
9	Anti-E	Maternal Plasma	E+	Yes
10	Anti-C	Amniotic Fluid	C-	No
11	Anti-K	Maternal Plasma	K-	No
12	Anti-K	Maternal Plasma	K+	Yes
13	Anti-K	Maternal Plasma	K-	No
14	Anti-D, Anti-C	Maternal Plasma	D-, C+*	Yes
15	Anti-c	Maternal Plasma	c-	No
16	Anti-c	Maternal Plasma	c+	Yes
17	Anti-D	Maternal Plasma	D-	No
18	Anti-c	Maternal Plasma	c+	Yes
19	Anti-K	Maternal Plasma	Inconclusive	Yes
20	Anti-D, Anti-C	Amniotic Fluid	D+, C-	Yes

*baby inherited hybrid RHD-CE-Ds gene from father (probably produces abnormal C antigen)

B. Crossmatch/Reference Laboratory

The Crossmatch/Reference Laboratory within Diagnostic Services at Canadian Blood Services provides transfusion medicine services (Crossmatch) for 25 hospitals in northern Alberta and 2 in the Northwest Territories that currently do not routinely perform these tests. Antibody investigation (Reference) services are provided for hospitals in Alberta, Northwest Territories, northeastern British Columbia and Lloydminster, Saskatchewan. Specimens from these sites are submitted for antibody identification, blood group discrepancy resolution, direct antiglobulin testing, fetal bleed screening and other serological testing.

Testing Performed

The Crossmatch/Reference Laboratory routinely performs the following tests:

- ABO/Rh blood type
- Screen for red blood cell antibodies
- Antibody identification, if antibodies are detected
- Crossmatches*
- Phenotyping (Patient and Donor Units)
- Direct Antiglobulin Tests
- Fetal Bleed Screens
- Other serological testing as required, as part of serological investigations

*Note: Tagged, crossmatched units are not issued to hospitals sending samples for antibody identification. These hospitals are responsible for performing their own crossmatches.

Our routine method for antibody screening and identification is by solid phase testing. Tube testing methods using PEG and LISS for enhancement are also utilized.

Stock and crossmatched blood components are distributed through the Crossmatch/Reference Laboratory to those hospitals which receive all of their transfusion medicine services from Canadian Blood Services. Hospitals which are referring specimens for additional testing and resolution receive all of their blood components through the Product Distribution area at Canadian Blood Services.

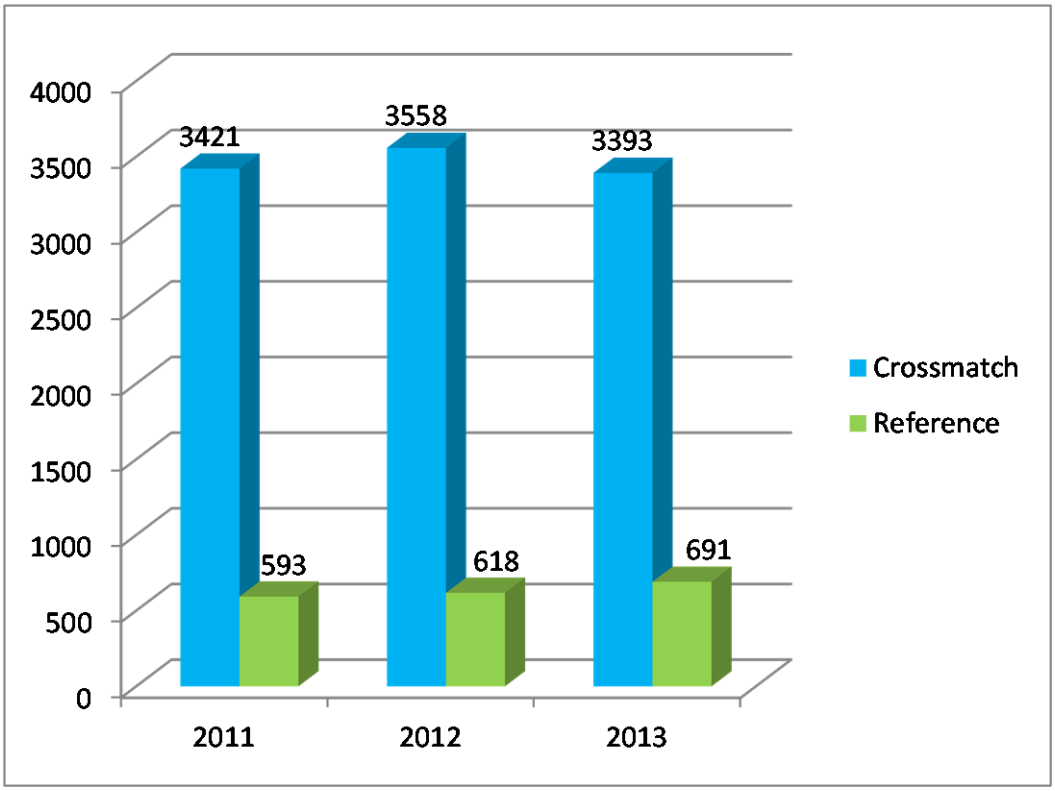
Specimens Tested

The total number of crossmatch specimens tested decreased by 6% in 2013 while the number of reference specimens increased by 8%. Changes were made so that four hospitals no longer send their crossmatches to us. The hospitals in Hinton and High Level are now performing all of their own crossmatches but referring problem crossmatches to us for antibody identification. Hinton is also performing crossmatches for the hospitals in Jasper and Edson. These changes came about because of increasing difficulties in shipping samples and blood between these sites and Edmonton. Having the hospitals take responsibility for crossmatching in their own areas means patients are able to receive the blood components they need in a shorter time frame.

Table 5: Crossmatch/Reference Specimen Testing Totals

Specimen Source	*Test Type*	*2011*	*2012*	*2013*
Crossmatch	Group and Screens/ Crossmatches	3421	3558	3393
Reference	Various tests requested	593	618	691
TOTAL		**4014**	**4176**	**4084**
Patients Tested		**N/Av**	**2416**	**2330**

Figure 3: Crossmatch/Reference Specimens Tested



Quality Indicators

Turn-Around-Times

In keeping with College of Physicians and Surgeons of Alberta (CPSA) laboratory accreditation requirements and to ensure timely reporting of patient results, Canadian Blood Services monitors turn-around times (TAT) from when the specimen is received at Canadian Blood Services in Edmonton to the time when the results are available.

Table 6: Turn-Around Time Criteria

Specimen Type	*Expected Turn-Around Time*	*% of Specimens Which Meet or Exceed Expected TAT*
Prenatal Specimens	72 hours	75%
Crossmatch Specimens	4 hours	85%
Reference Specimens	24 hours	85%

Figure 4: Turn-Around Times – Reference Specimens

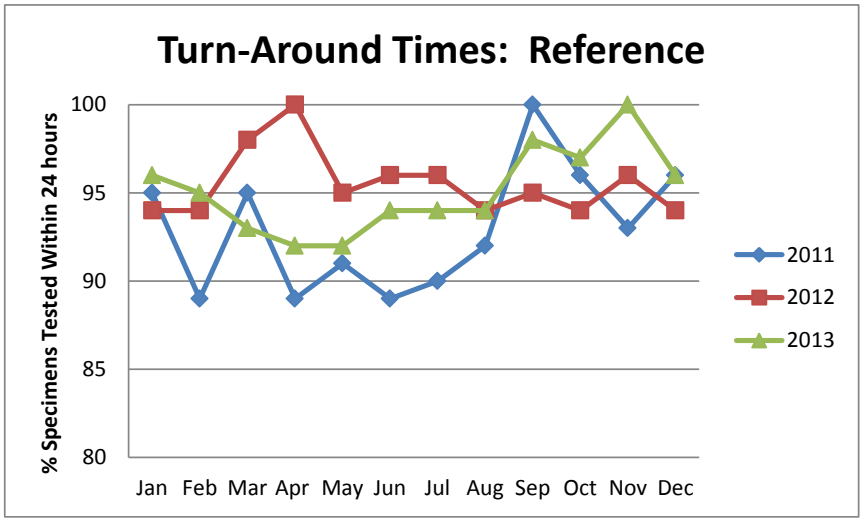


Figure 5: Turn-Around Times - Crossmatch Specimens

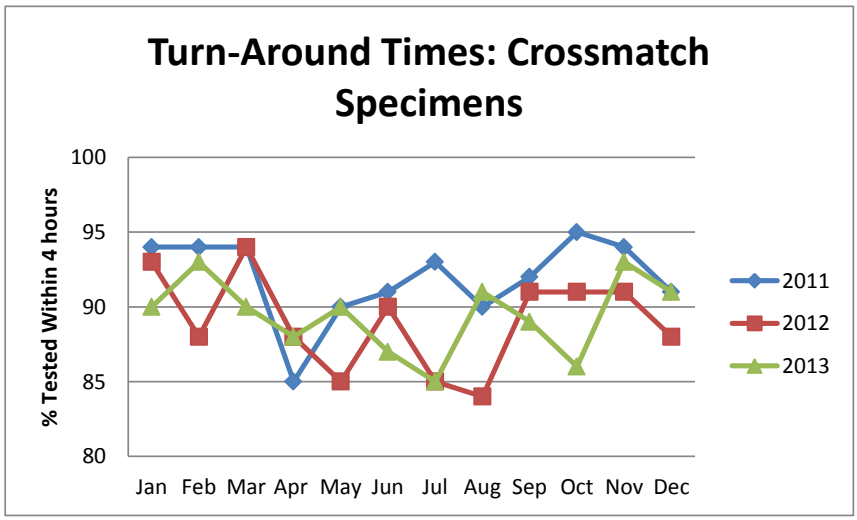
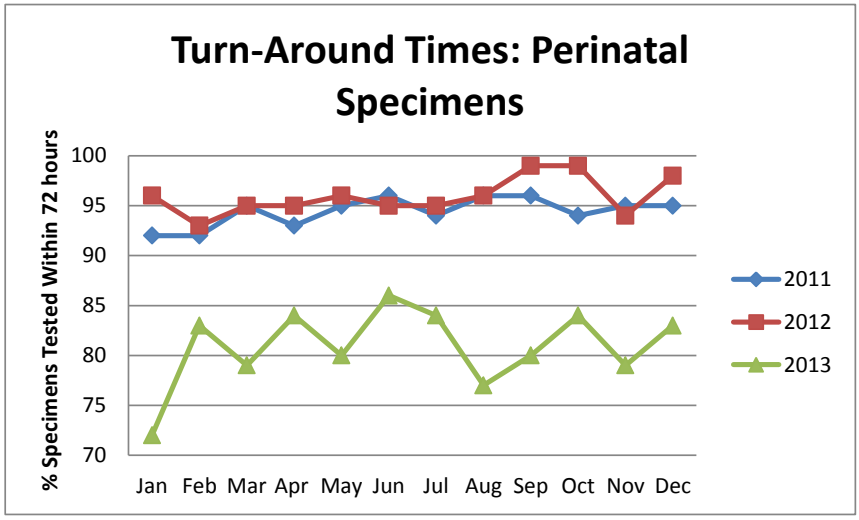


Figure 6: Turn-Around Times – Prenatal Specimens



We are consistently meeting or exceeding our turn-around time target of 85% of Crossmatch and Reference specimens completed within the established time frame for that specimen type. There has been a decrease in the percentage of Perinatal samples tested within 72 hours. This is because we made changes to the Prenatal testing schedule in January 2013. Testing that was performed in the late afternoon/early evening is now being done on the following day shift. Samples received on Friday are not tested until Monday morning. Results are faxed out on Monday afternoons as was done previously so there was really no change for physician offices.

Rejected Specimens

Each time a specimen is rejected, a reason for rejection is entered into our Laboratory Information System. This data is then retrieved and analysed on a quarterly basis. The number of rejected specimens is quite low for Crossmatch/Reference samples which are coming from hospitals and for Perinatal samples which are primarily collected at community collection sites. The Diagnostic Services laboratory is following the provincial specimen rejection guidelines for Alberta.

The reasons for rejecting specimens in the Crossmatch/Reference and the Perinatal laboratories are somewhat different. More crossmatch specimens are rejected because of problems with the requisition missing critical information such as the Blood Bank Identification number, PHN or phlebotomist signature. The increased rates seen in Quarter 2 and Quarter 3 seemed to be related to new staff in hospital laboratories.

For perinatal specimens, the most common reason for rejecting a sample is that the sample is a duplicate. Samples are rejected if another sample from the patient was tested within the previous 96 hours. Often a duplicate sample is collected when the patient has seen their family physician and then sees their obstetrician shortly after. We do ensure that the report from the initial testing is sent to the second physician making the request. Health care professionals can access Canadian Blood Services reports for Alberta patients on Netcare, Alberta's Electronic Health Record.

Figure 7: Quarterly Rejection Rates

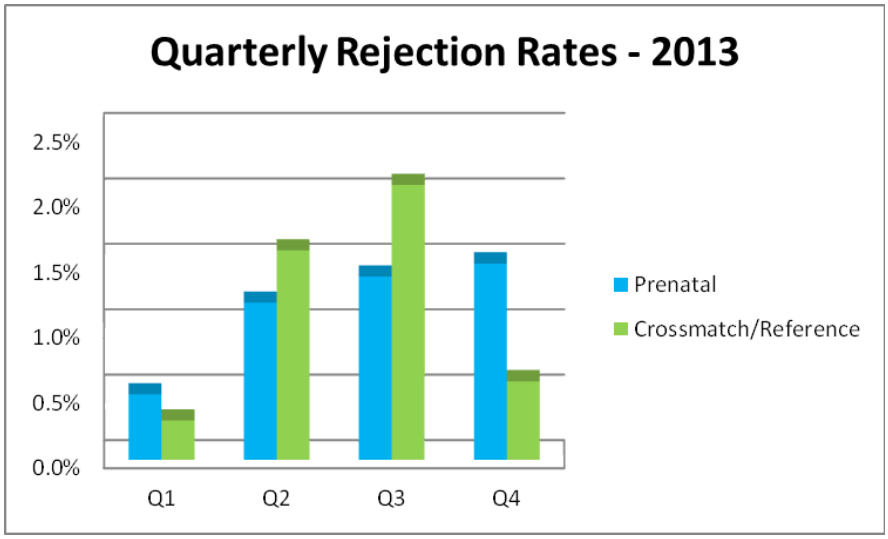
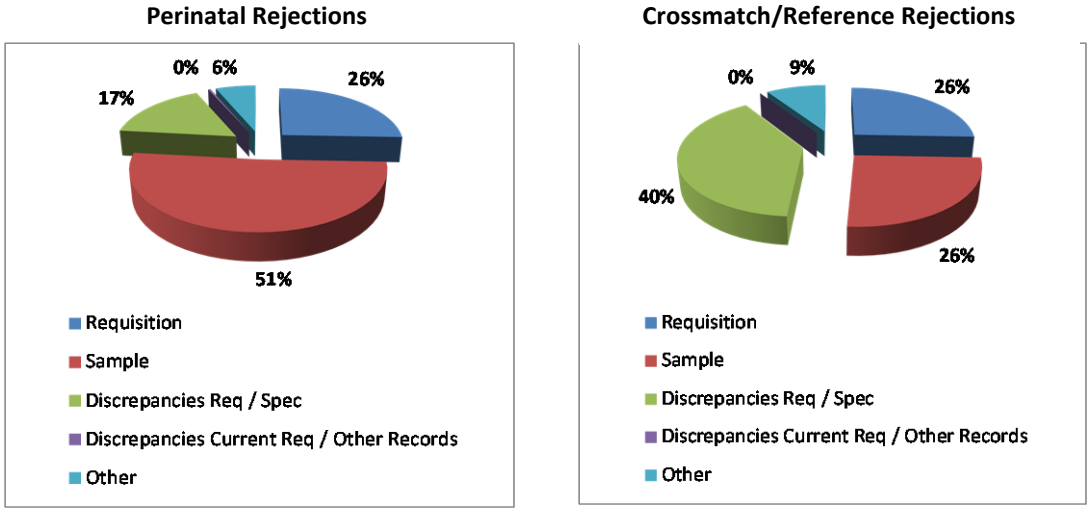


Figure 8: Rejection Reasons



D. Accomplishments of 2013 and Goals for 2014

General

Red Cell Antigen Genotyping

Immucor's BioArray™ BeadChip™ testing system was installed and validation of two test kits (one for red blood cell antigen genotyping and the other for RHD genotyping) was completed in early 2014. The pilot project on red cell antigen genotyping will begin in June 2014. Red cell genotyping will be used to help to resolve complex red blood cell antibody investigations for patients who require blood transfusions and for pregnant mothers with unclear RhD status to determine Rh Immune Globulin eligibility.

Business Continuity Planning

Canadian Blood Services continued the process of developing business continuity plans for all sites. The plan for the Edmonton facility was started in 2013 and should be completed in 2014. The contingency plan for Diagnostic Services will see Alberta Health Services sites in Edmonton provide the back-up testing for the crossmatch and reference specimens with support from Canadian Blood Services staff to get the blood to the rural sites. Testing of prenatal specimens will be moved to the Canadian Blood Services site in Vancouver but still be done by some of our Edmonton staff.

Perinatal Laboratory

Fetal Genotyping Using Maternal Plasma

The process of sending maternal plasma to the Lab in Bristol, England, for isolation of fetal DNA for fetal genotyping was developed and successfully implemented. This testing makes fetal genotyping much more available and significantly less risky for the mother and her fetus. As such, we anticipate the use of this service to increase over the next few years. Occasionally, there will be a need to submit another sample, specifically if fetal DNA is not detected or when there is a negative result when detecting genes in the Kell system. Since continued follow-up is required when there is either a positive or negative result for the Kell system antigens, we are now recommending only sending samples after 28 weeks.

Perinatal Advisory Committee

The Diagnostic Services Director, Medical Directors, Managers and Supervisors from all of the Canadian Blood Services Diagnostic Laboratory sites (in Vancouver, Edmonton, Regina, Winnipeg and Toronto) meet once annually to discuss operational issues and 'best practice' approaches for serological and perinatal laboratory testing. In discussions where expert advice is required, guest speakers are invited to provide input and direction. Working groups are set up as required to investigate specific issues and bring recommendations forward. Input is obtained from relevant stakeholders on planned policy changes.

Anecdotally, there had been some concern about anti-c causing HDFN even at low titres. As such, we had begun to recommend referral to the Maternal-Fetal Medicine clinic for all anti-c. Based on a retrospective study of clinical outcomes for antibodies other than anti-D which was done in the Edmonton area, the critical titre value of 16 for the other common antibodies appears to be valid. We have stopped referring patients with anti-c to the Maternal-Fetal Medicine clinic unless a critical titre of $\geq$16 is reached.

A review of literature regarding anti-M revealed that, although this antibody is rarely implicated in HDFN, it may cause suppression of fetal erythropoiesis and late onset anemia (Trans Med Rev 2014: 28:1-6). The Perinatal Advisory Committee recommended that a comment be added to reports of anti-M advising that the baby be monitored for symptoms of late onset anemia for up to 2 months of age.

Some current initiatives being undertaken by group members include:

- Monitoring the international developments in providing screening for Neonatal Alloimmune Thrombocytopenia (NAIT)
- Monitoring the international developments in assessment of fetal RHD status based on analysis of cell-free fetal DNA (cff DNA) in mother's plasma as a basis for determining RhIG eligibility (targeted RhIG prophylaxis). This approach is becoming the standard in many European countries.

Crossmatch/Reference Laboratory

Use of Galileo Neo in our Crossmatch/Reference Lab

Our second Galileo Neo was validated and implemented by mid-July 2013. We now have automated testing with direct resulting into our Lab Information System (LIS).