Canadian Blood Services
Société canadienne du sang

DIAGNOSTIC SERVICES

Saskatchewan

YEAR IN REVIEW

JANUARY – DECEMBER 2015

Diagnostic Services “Year in Review” statistics are based on a January to December calendar year. The calendar year provides better correlation with Health Canada birth statistics.

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PERINATAL LABORATORY

The Perinatal Laboratory within Diagnostic Services at Canadian Blood Services provides diagnostic testing of pregnant women for blood type and red blood cell antibodies. Results from this screening assist physicians, midwives and nurse practitioners in ensuring the appropriate management of a pregnancy for both the mother and baby.

A. 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 Identification referrals
- Antibody Titre, if a clinically significant antibody is identified
- Phenotyping
- Direct Antiglobulin Test for detection of HDFN (Hemolytic Disease of the Fetus/Newborn)

B. Testing 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 patient is at increased risk of allo-immunization (e.g. previous transfusion, fetal trauma or procedure, IV drug use).

Mothers – Antibody Present If the antibody is known to cause HDFN, it is recommended that specimens be submitted again at 18-20 weeks, then every four weeks until 32 weeks gestation. Samples should then be submitted every two weeks until delivery.

Newborns (Cords) Cord blood or neonatal samples may be submitted for testing. ABO/Rh and direct antiglobulin testing is performed on the samples.

Partners When a woman has an antibody capable of causing HDFN, specimens from the partner will be requested for ABO/Rh and antigen phenotyping. This will assist in assessing the probability of the baby being affected by the antibody. Partners' specimens may also be tested to assess Rh Immune Globulin (RhIG) eligibility of Rh negative mothers.

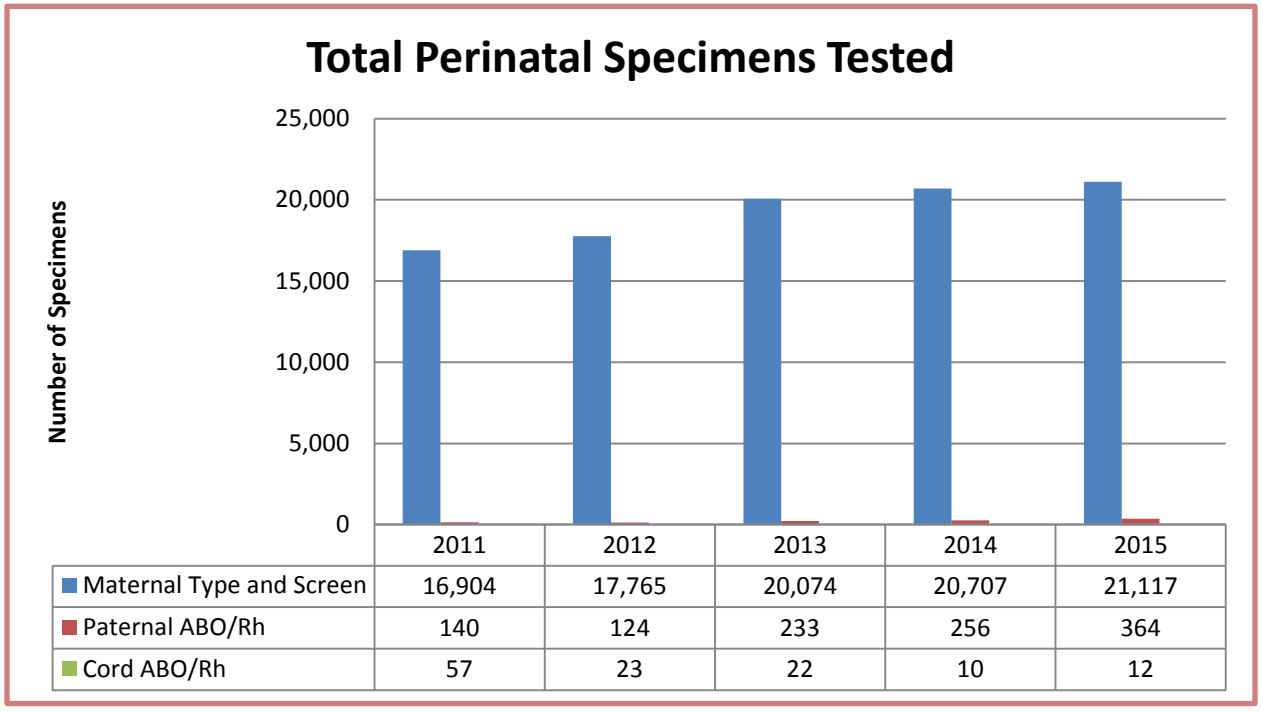
C. Specimens Tested

The data includes all women tested, including referrals.

Table 1: Perinatal Specimens Tested

Specimen	Test Type	2011	2012	2013	2014	2015
Maternal	Type and Screen	16,904	17,765	20,074	20,707	21,117
Paternal	ABO/Rh	140	124	233	256	364
Cord	ABO/Rh	57	23	22	10	12
Total # of Specimens Tested		**17,101**	**17,912**	**20,329**	**20,973**	**21,493**
Total # of Patients Tested		**N/Av**	**14989**	**16925**	**17,450**	**17,631**

Figure 1: Total Perinatal Specimens Tested



D. Antibodies Identified

In 2015, a total of 159 antibodies were reported (see *Table 2*). This is slightly lower than 2014. One hundred and twenty one women had antibodies identified during their pregnancies, of these; twenty-three women had multiple antibodies.

Antibodies identified were considered to be clinically significant if they have been reported to cause HDFN. The most common clinically significant antibodies identified were: anti-E, anti-K, anti-D, anti-C, and anti-c which together represented 70% of the total antibodies identified.

Titres for 8 of the clinically significant antibodies increased from non-critical to critical levels during the pregnancy with a total of 22 antibody titres at critical levels (see *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 High Risk Fetal Assessment Clinic for further follow-up and monitoring during pregnancy.

Table 2: Total Number of Perinatal Antibodies Detected

Maternal Antibodies Identified (Including Passive D)

Clinically Significant Antibodies - Antibody	2011	2012	2013	2014	2015
Anti-D	11	12	9	16	10
Anti-C	5	8	10	14	10
Anti-E	25	32	33	39	38
Anti-c	10	11	3	16	12
Anti-e	2	4	2	3	5
Anti-Cw				1	3
Anti-M	12	14	12	13	10
Anti-K	24	20	17	24	30
Anti-S	5	3	4	6	3
Anti-s				1	1
Anti-Fy[a]	5	3	2	9	6
Anti-Fy[b]	1	1	1	2	1
Anti-Jk[a]	6	6	10	6	8
Anti-Jk[b]	3	1	1	4	3
Anti-Lua				1	0
Anti-Lub	1				0
Anti-Kp[a]				2	0
Anti-G	1	1			1
Anti-Cob					1
Anti-Wra					1

Passive Anti-D	N/Av	156	123	156	178
Clinically Insignificant Antibodies - Antibody	2011	2012	2013	2014	2015
Anti-Le[a]	9	7	10	12	14
Anti-Le[b]	1	1	1	2	2
Anti-N			2	1	

Table 3: Perinatal Patient Antibody Titres

Antibody	Critical Level	Non-Critical Level	Non-Critical to Critical
Anti-D	6	6	2
Anti-C	1	6	0
Anti-E	3	31	3
Anti-c	1	8	1
Anti-e	0	1	0
Anti-DC	0	1	0
Anti-DE	0	1	0
Anti-Ec	4	4	2
Anti-Ce	0	2	0
Anti-G	0	1	0
Anti-K	5	8	0
Anti-Fya	2	3	0
Anti-Fyb	0	1	0
Anti-Jka	0	4	0
Anti-Jkb	0	2	0
Anti-M	0	3	0
Anti-S	0	2	0
Anti-s	0	1	0

Figure 2: Total Number of Perinatal Antibodies

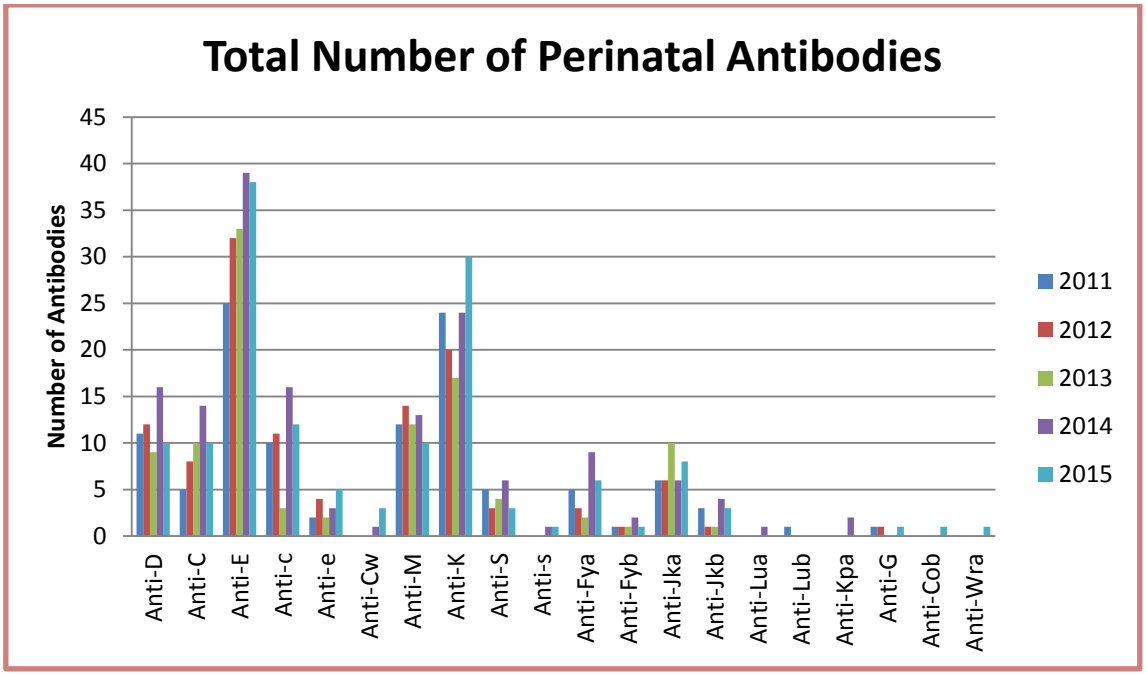


Figure 3: Frequency of Clinically Significant Antibodies

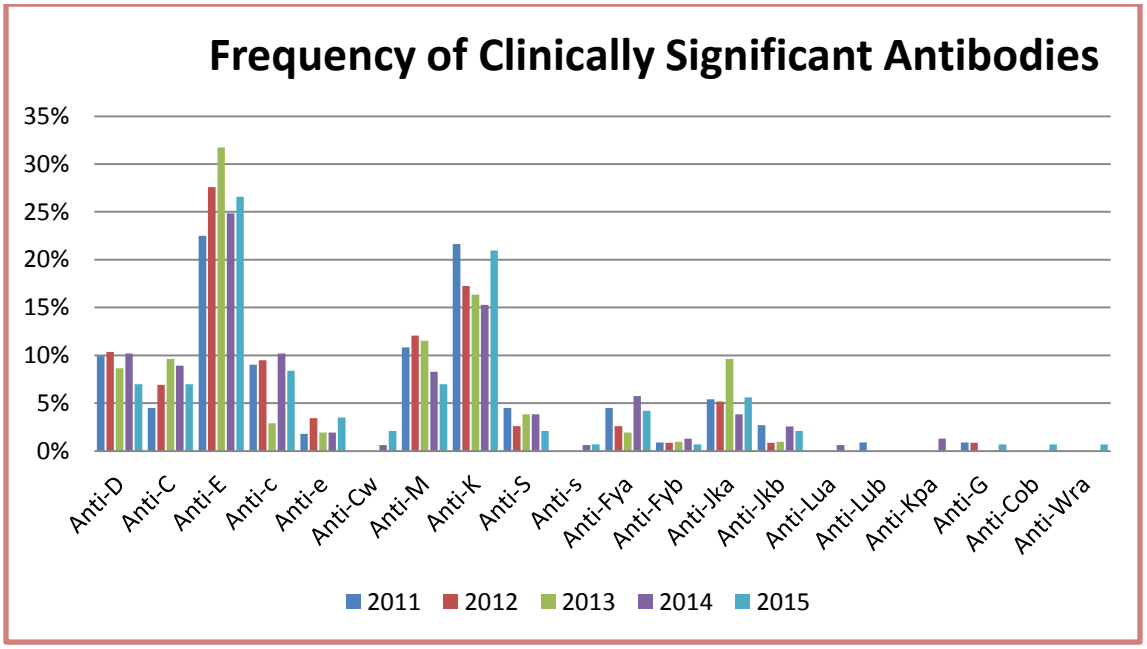


Table 4: Combination Antibodies

Antibodies	Number in 2015
Anti-C Anti-D	3
Anti- C Anti-D Anti-E	1
Anti-C Anti-D Anti-G	1
Anti-C Anti-e	2
Anti-c Anti-E	4
Anti-c Anti-E Anti-Fya Anti-K Anti-s	1
Anti-c Anti-E Anti-Jka	1
Anti-c Anti-Fya	1
Anti-C Anti-K	1
Anti-Cw Anti-K	1
Anti-E Anti-Jkb	1
Anti-E Anti-K	2
Anti-E Anti-S	1
Anti-K Anti-Lea	1
Anti-Lea Anti-Leb	1
Anti-K Anti-M	1

CROSSMATCH / REFERENCE LABORATORY

The Diagnostic Services Crossmatch/Reference Laboratory provides transfusion medicine services and reference testing to 38 hospitals within 11 Health Regions in Saskatchewan.

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
- Crossmatch, electronic and serological
- Phenotyping (patient and donor units)
- Transfusion Reaction Investigation
- Direct Antiglobulin Test
- Elution and Absorption

Antibody screening and identification is routinely performed by tube testing using PEG for enhancement.

Crossmatched blood components are distributed through the Diagnostic Services Laboratory to those hospitals which receive all of their transfusion medicine services from Canadian Blood Services. Hospitals

which provide transfusion medicine services directly receive all of their blood components through the Product and Hospital Services area at Canadian Blood Services.
As a Reference Laboratory, the Crossmatch Laboratory performs complex antibody investigations and distributes crossmatch compatible (or least incompatible) red cell units.

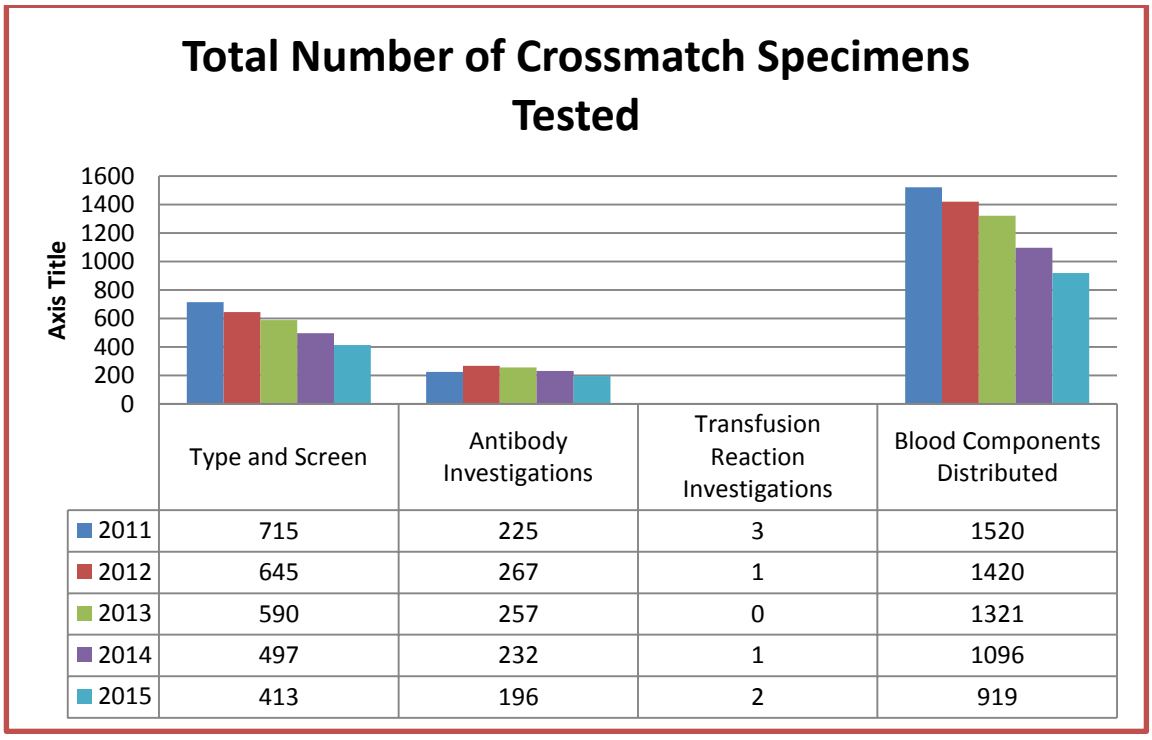
A. **Specimens Tested**

The data in this report reflects a calendar year period to enable better correlation to other government statistical data. The total number of crossmatch specimens tested has shown a slight decrease over the past four years.

Table 5: Crossmatch/Reference Specimens Tested

Specimen Type	Test Type	2011	2012	2013	2014	2015
Crossmatch /Reference	Type and Screen	715	645	590	497	413
	Antibody Investigations	225	267	257	232	196
	Transfusion Reaction Investigations	3	1	0	1	2
	Blood Components Distributed	1520	1420	1321	1096	919
Test Totals (excluding components distributed)		**943**	**913**	**847**	**730**	**611**
Number of Patients Tested		**N/Av**	**346**	**328**	**262**	**242**

Figure 4: Total Crossmatch Specimens Tested



B. Antibodies Identified

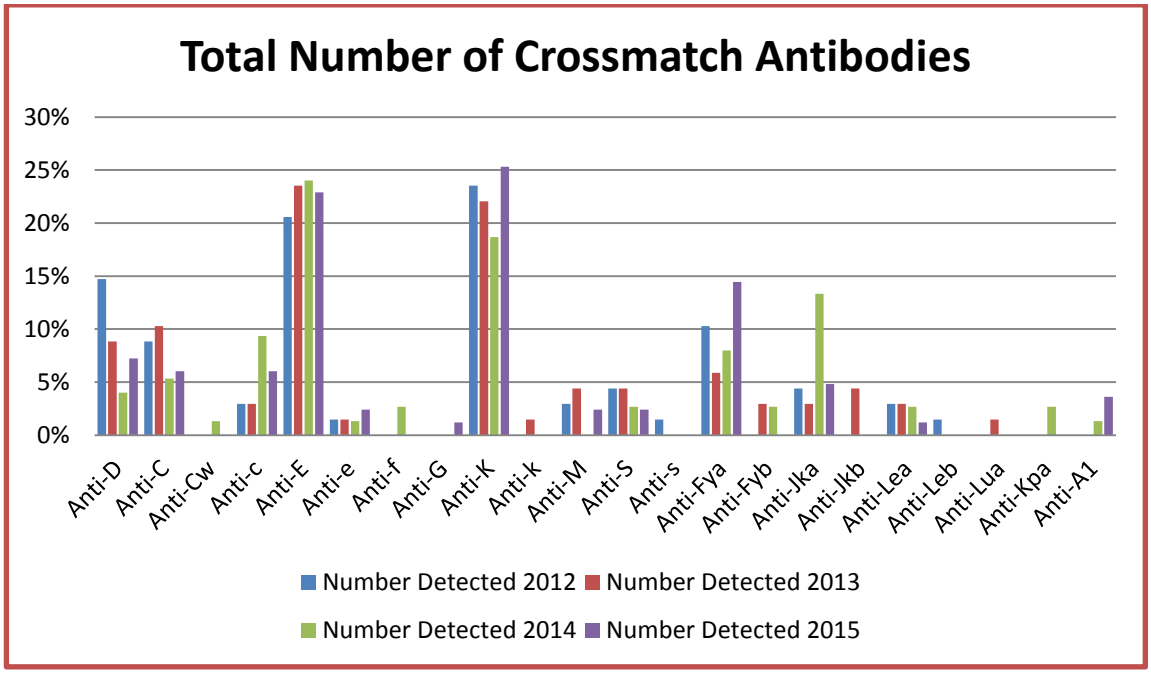
In 2015, a total of 83 antibodies were reported (see *Table 6*). The total number of antibodies detected is slightly higher than in 2014, but the distribution of the most common antibodies remains consistent.

Antibodies identified were considered to be clinically significant if they have been reported to cause acute or delayed hemolytic transfusion reactions. The most common clinically significant antibodies identified were: anti-E, anti-K, anti-Jk^{a}, anti-D and anti-Fy^{a} (see *Figure 5*) which together represented 69% of the total antibodies identified.

Table 6: Total Number of Crossmatch Antibodies Detected

Antibody	Number Detected 2012	Number Detected 2013	Number Detected 2014	Number Detected 2015
Anti-D	10	6	3	6
Anti-C	6	7	4	5
Anti-Cw			1	
Anti-c	2	2	7	5
Anti-E	14	16	18	19
Anti-e	1	1	1	2
Anti-f			2	
Anti-G				1
Anti-K	16	15	14	21
Anti-k		1		
Anti-M	2	3		2
Anti-S	3	3	2	2
Anti-s	1			
Anti-Fya	7	4	6	12
Anti-Fyb		2	2	
Anti-Jka	3	2	10	4
Anti-Jkb		3		
Anti-Lea	2	2	2	1
Anti-Leb	1			
Anti-Lua		1		
Anti-Kpa			2	
Anti-A1			1	3
Total	68	68	75	83

Figure 5: Total Number of Crossmatch 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. We have also begun to refer specimens to the International Blood Group Reference Laboratory (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 (ie 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.

Based on the following testing algorithm patients with a serologically variable Rh D typing results may have a genetic testing for the RHD gene. For 2015, the following results were obtained in patients using one of the two Red Cell antigen genotyping platforms available at CBS:

Figure 6: Rh D Testing Algorithm

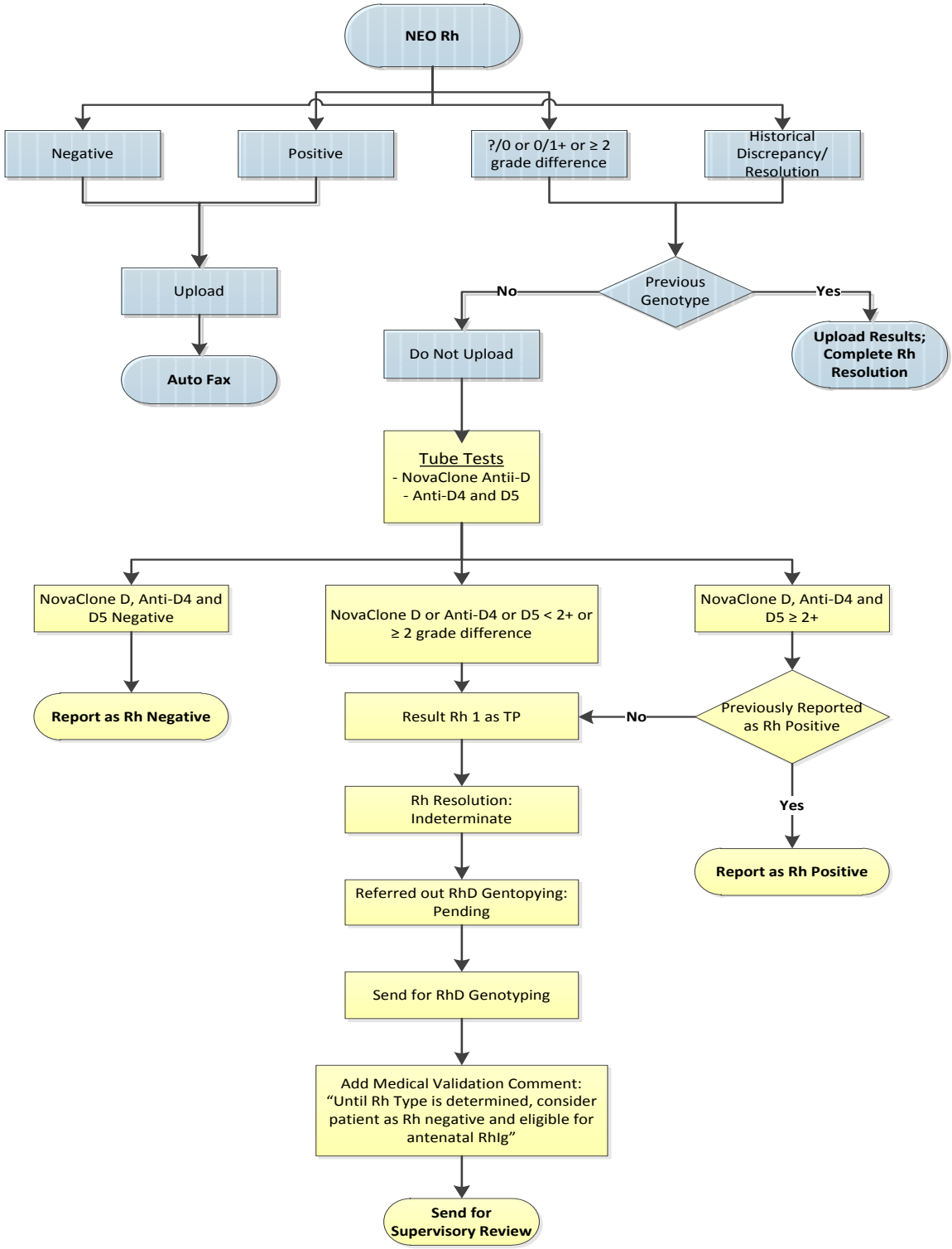


Table 7: Patient # - RHD Type/Result

Patient	RHD Genotype	Predicted Phenotype	RHD Sequencing	Rh Group
1	Sample does not contain any of the RHD polymorphisms interrogated by the kit	D Variant	No	Negative
2	Sample does not contain any of the RHD polymorphisms interrogated by the kit	D Variant	No	Negative
3	Sample does not contain any of the RHD polymorphisms interrogated by the kit	D Variant	No	Negative
4	Sample does not contain any of the RHD polymorphisms interrogated by the kit	D Variant	No	Negative
5	Weak D Type 4.0 or 4.3 (RHD*09.03 or RHD*09.05)	Weak D	No	Negative
6	Sample does not contain any of the RHD polymorphisms interrogated by the kit	D Variant	No	Negative
7	Sample does not contain any of the RHD polymorphisms interrogated by the kit	D Variant	No	Negative
8	Sample does not contain any of the RHD polymorphisms interrogated by the kit	D Variant	No	Negative
9	Sample does not contain any of the RHD polymorphisms interrogated by the kit	D Variant	No	Negative
10	Sample does not contain any of the RHD polymorphisms interrogated by the kit	D Variant	No	Negative
11	Sample does not contain any of the RHD polymorphisms interrogated by the kit	D Variant	No	Negative
12	Sample does not contain any of the RHD polymorphisms interrogated by the kit	D Variant	No	Negative
13	DAR (RHD*09.01) weak D type 4.2	Weak D	No	Negative
14	Sample does not contain any of the RHD polymorphisms interrogated by the kit	D Variant	No	Negative
15	DNA sequencing of RHD Exon 9. RHD*1187G	Weak D	Exon 9	Negative
16	RHD*weak D type 4.0	Partial/Weak D	Exon 6	Negative

17	DNA sequencing of RHD Exon 9. RHD*1187G	Weak D	Exon 9	Negative
18	DNA sequencing of RHD Exon 9. RHD*1187G	Weak D	Exon 9	Negative
19	Weak D Type 3	Weak D	not in file	Positive
20	DNA sequencing of RHD Exon 9. RHD*1187G	Weak D	Exon 9	Negative
21	Sample does not contain any of the RHD polymorphisms interrogated by the kit	D Variant	No	Negative
22	Sample does not contain any of the RHD polymorphisms interrogated by the kit	D Variant	No	Negative
23	Weak D Type 3 (RHD*01W.3)	Weak D	No	Positive
24	Weak D Type 3 (RHD*01W.3)	Weak D	No	Positive
25	Sample does not contain any of the RHD polymorphisms interrogated by the kit	D Variant	No	Negative
26	Sample does not contain any of the RHD polymorphisms interrogated by the kit	D Variant	No	Negative
27	Sample does not contain any of the RHD polymorphisms interrogated by the kit	D Variant	No	Negative
28	DOL or DOL2 (RHD*12.01 or RHD-12.02)	D Variant	No	Negative
29	Sample does not contain any of the RHD polymorphisms interrogated by the kit	D Variant	No	Negative
30	Sample does not contain any of the RHD polymorphisms interrogated by the kit	D Variant	No	Negative
31	Sample does not contain any of the RHD polymorphisms interrogated by the kit	D Variant	No	Negative
32	Sample does not contain any of the RHD polymorphisms interrogated by the kit	D Variant	No	Negative
33	Weak D Type 3 (RHD*01W.3)	Weak D	No	Positive
34	Weak D Type 4.1 (RHD*09.04)	Weak D	No	Negative
35	Possible D Variant/RHD psi(RHD*04N.01)	D Variant	No	Negative
36	Weak D Type 3 (RHD*01W.3)	Weak D	No	Positive

QUALITY INDICATORS

The laboratories monitor many quality indicators and the two which are most relevant to this document are turnaround times and rejected specimens which are presented below.

A. Turnaround Times

To ensure timely reporting of patient test results, Canadian Blood Services monitors turnaround time (TAT) from when the specimen is received at Canadian Blood Services in Winnipeg to the time when the results are available. Since monitoring of this quality indicator began in 2008, the percentage of specimens has consistently exceeded the predefined TAT threshold. Samples whose testing exceeds the expected TAT are usually those where clinically significant antibodies are detected or where difficulty in finding compatible blood is encountered.

Table 8: Turnaround Time – Routine Criteria by Specimen Type

Specimen Type	Expected Turnaround Time	Expected % of Specimens Which Meet or Exceed Expected TAT
Routine Perinatal Specimens	72 hours	85%
Routine Crossmatch Specimens	24 hours	85%
Reference Specimens	72 hours	75%

Figure 7: Perinatal Routine TAT

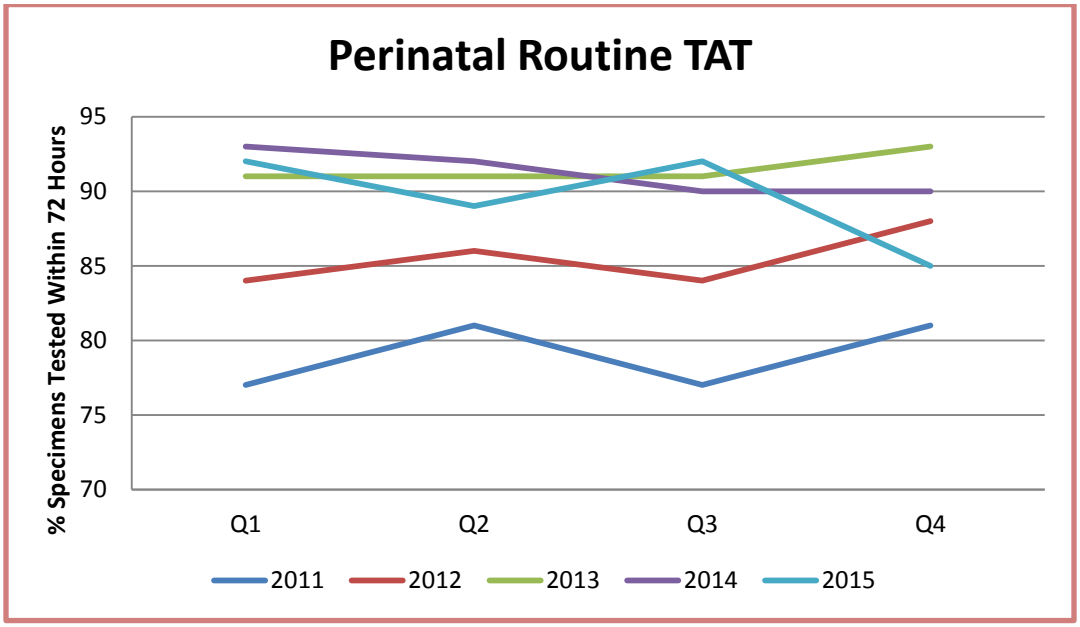


Table 9: Turnaround Time – Routine Perinatal Specimens

Turn Around Time (TAT)	2011	2012	2013	2014	2015
% of Specimens Tested within 72 hours	89%	88%	88%	87%	90%
% of Specimens Tested > 72 hours	11%	13%	13%	13%	10%

Figure 8: Crossmatch Routine TAT

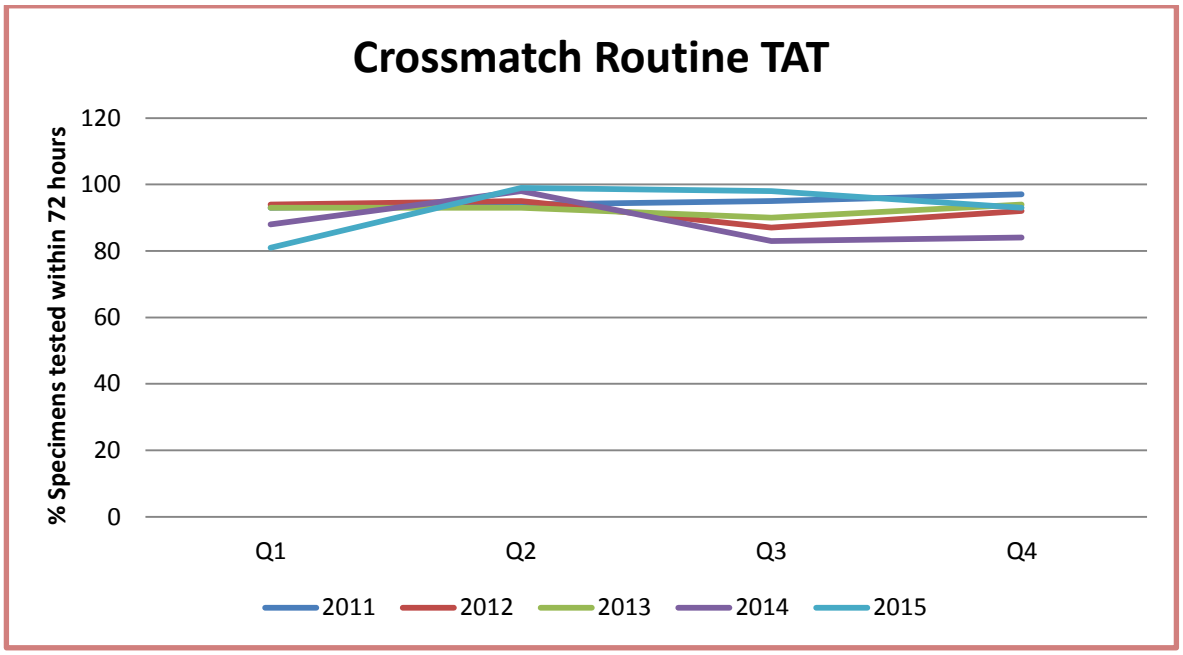


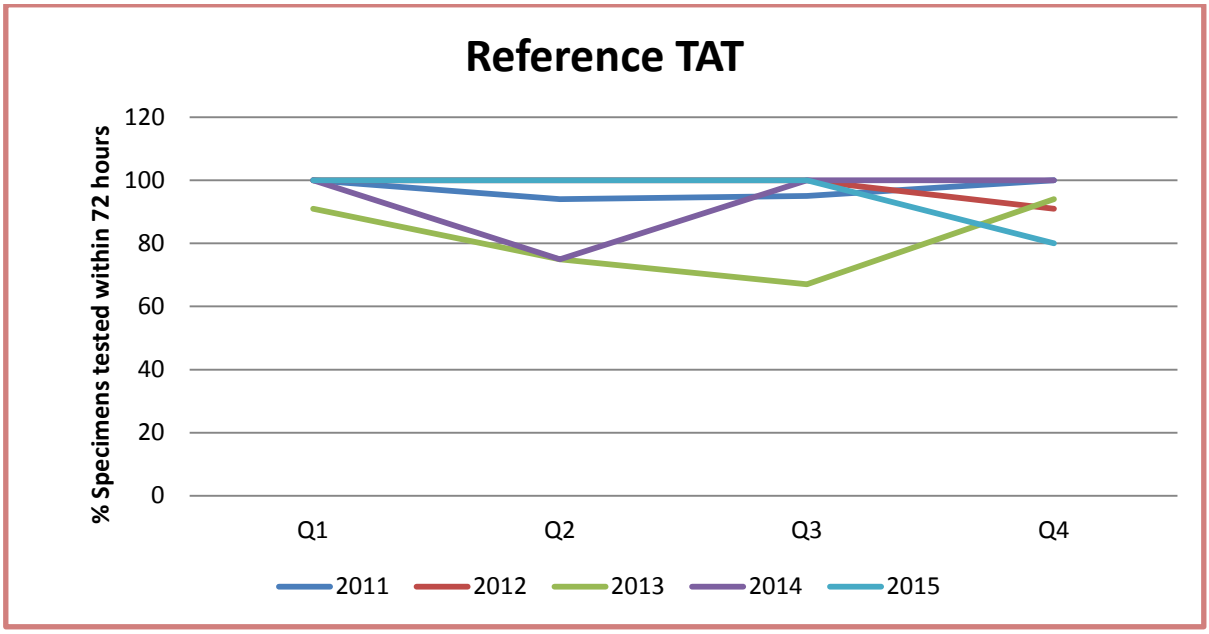
Table 10: Turnaround Time – Routine Crossmatch Specimens

Turn Around Time (TAT)	2011	2012	2013	2014	2015
% of Specimens Tested within 72 hours	94%	93%	92%	91%	93%
% of Specimens Tested > 72 hours	6%	7%	8%	9%	7%

Table 11: Reference TAT

Turn Around Time (TAT)	2011	2012	2013	2014	2015
% of Specimens Tested within 72 hours	100%	100%	81%	92%	95%
% of Specimens Tested > 72 hours	2%	0%	2%	1%	5%

Figure 9: Turnaround Time – Reference Specimens



B. Rejected Specimens

Each time a specimen is rejected, a reason for rejection is entered into our laboratory information system (LIS). This data is then retrieved and analyzed on a quarterly basis.

As described in *Table 12*, the reasons for rejecting specimens in the Perinatal Laboratory are distributed similarly between problems with requisitions and discrepancies between the requisition and the specimen. Also, a number of samples fell outside of the testing criteria, having been tested within the current pregnancy (Other category). Average rejection rates have continued to decrease from 2.6% in 2012 to 1.7% in 2015 which correlates with increased efforts to contact customers and educate them on acceptable labelling criteria.

Table 13 describes the reasons for rejecting specimens in the Crossmatch Laboratory. The majority are due to test cancellation (Other category). Problems with requisition and specimen labelling are rare occurrences.

Table 12: Quarterly Rejection Rates – Perinatal Specimens

Rejection Category	Q1	Q2	Q3	Q4
Requisition	23	18	23	26
Specimen	23	15	21	21
Discrepancies Between Requisition & Specimen	22	19	14	35
Discrepancies Between Current Requisition & Historical Records	2	4	3	0
Other	23	18	26	27
Total # specimens rejected	93	74	87	109
Total # specimens received	5117	5712	5157	5472
Rejections as a % of total	**1.8%**	**1.3%**	**1.7%**	**2.0%**

Figure 10: Perinatal Rejection Reasons

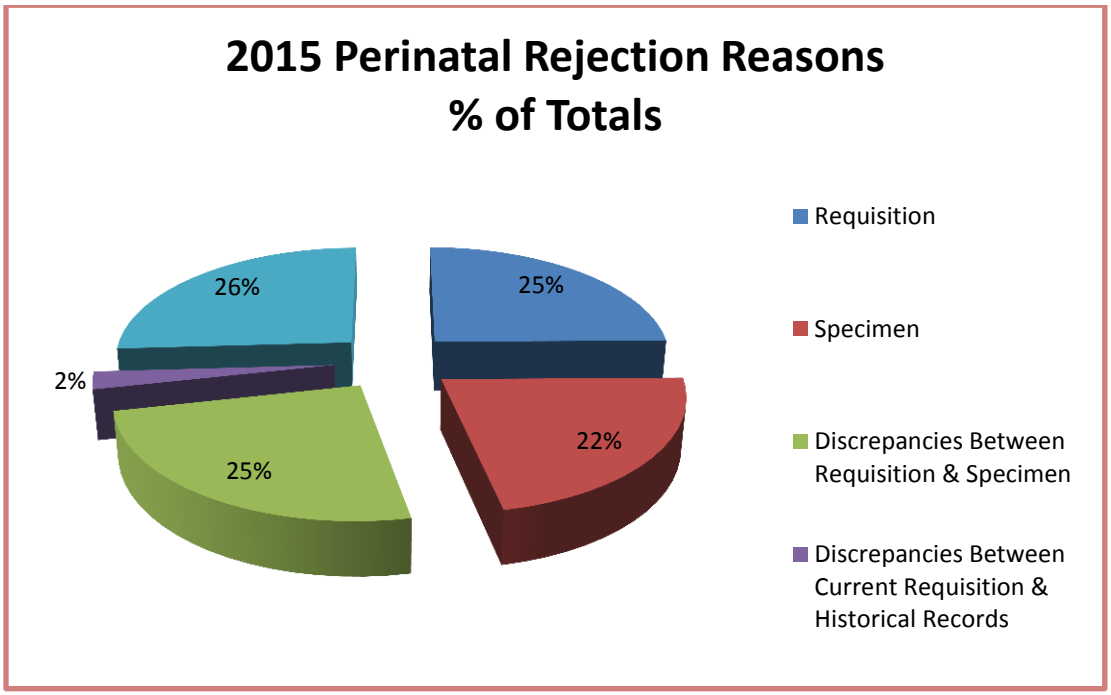


Table 13: Quarterly Rejection Rates – Crossmatch Specimens

Rejection Category	Q1	Q2	Q3	Q4
Requisition	0	0	0	0
Specimen	0	0	0	0
Discrepancies Between Requisition & Specimen	0	0	0	0
Discrepancies Between Current Requisition & Historical Records	0	0	0	0
Other	2	0	1	3
Total # specimens rejected	2	0	1	3
Total # specimens received	90	105	58	98
Rejections as a % of total	**2.2%**	**0.0%**	**1.7%**	**3.1%**

ACCOMPLISHMENTS IN 2015

A. Automated Testing Instrument Upgrade

In 2015 all Diagnostic Services sites (Vancouver, Edmonton, Regina and Winnipeg) participated in the post implementation review of the common algorithm for the investigation of positive antibody screens obtained on the Galileo Neo.

B. Genotyping – Red Cell

Canadian Blood Services is able to provide red cell antigen genotyping services through our National Immunohematology Reference Laboratory (NIRL). A process for the referral of perinatal and pre-transfusion specimens to NIRL for genotyping was developed and implemented. This service is used to aid in resolving complex immunohematology cases. Molecular testing combined with hemagglutination testing can provide better resolution to serological problems and guide patient transfusion requirements in some circumstances, in particular for sickle cell patients and patients with frequent transfusion requirements.

C. Perinatal Advisory Committee

The PNAC held their annual meeting on April 29 and 30, 2015 in Edmonton. Attendees included the Director, Testing, the Associate Director, Diagnostic Services and the Associate Medical Director, Clinical Services for CBS. The Medical Officers and Managers for the CBS Diagnostic Services Laboratories across the country and representatives from the CBS National Reference Laboratory (NIRL) in Ottawa as well as guests from user hospitals were also in attendance. The meeting included an overview of Diagnostic

Services activities over the past year, and discussion of procedures and quality issues affecting these laboratories.

Highlights of the two day meeting include the following:

- Human platelet antigen and antibody testing has been consolidated in the CBS Platelet Immunology Laboratory in Winnipeg and subsequent to this, an increase in test volumes has been observed. This laboratory has obtained College of American Pathologists (CAP) accreditation.
- Roll-out of the Trace Line hospital module has been completed in Manitoba.
- All testing services operating under the CBS umbrella have been consolidated into a single management structure.
- Genotyping using the Immucor BioArray BeadChip system was implemented in June 2014. Discussion occurred around the reporting and management of patients with weak D types 4.0, 4.1 and 4.3 in the context of a recent article by S. G. Sandler on behalf of the AABB/CAP Working Group on RHD Genotyping (Sandler SG et al. Transfusion March 2015; 55:680-689).
- Discussion occurred around expanding availability of testing for fetal DNA using maternal blood samples to provinces outside Alberta where this process is already in place.
- Report comments for anti-M in pregnancy was discussed in light of a recent article suggesting that this antibody rarely causes severe hemolytic disease of the fetus and newborn (HDFN) and/or delayed anemia in affected infants, particularly with patients of Asian extraction (Yasuda H et al. Transfusion Medicine Reviews 2014; 28: 1-6).

GOALS FOR 2016

A. Restructure Laboratory Services

Participate with MOH, 3S Health and Regional Health Authorities in discussions related to restructuring provincial laboratory services. Discussions will include transfer of the crossmatch services to RHAs and potential relocation of the perinatal testing program.

B. Perinatal Advisory Committee

The annual Perinatal Advisory Committee meeting for 2016 is planned for June 13 and 14 2016 in Winnipeg MB. The PNAC meeting will be followed by an Educational Event sponsored by Grifols. Attendees will include Laboratory Directors, Associate Directors and Managers as well as perinatal supervisory staff and laboratory physicians who oversee perinatal testing. We will also welcome some hospital colleagues, both technologists and physicians, who are involved with perinatal testing laboratories. Ongoing work on standardization among our laboratories is a theme for this year. Our meeting plan and ongoing work plan for the remainder of 2016 will include:

- Discussion and consensus on appropriate follow up for perinatal patients with inconclusive antibodies.
- Planning for investigation of patients with possible antibodies to low prevalence antigens in the perinatal setting. We will discuss the development of a standard “low prevalence” panel of cells that will allow for investigation of antibodies to low prevalence antigens which may be clinically significant in pregnancy.

- Discussion and consensus on the timing of repeat samples for patients with clinically significant or potentially significant antibodies in the perinatal setting.
- We will discuss the functionality of our standardized antibody investigation algorithm, including any necessary changes following one year of use.
- We will optimize and standardize the use of our algorithm used for RHD genotyping in perinatal patients with weak or variable Rh D serological typing.
- We will discuss the optimal serological evaluation for anti G, especially in the presence of passive anti D.
- We will discuss the results of an audit of Kell negative donor unit availability in transfusion of Kell negative (or Kell unknown) females of child bearing potential.
- We will have some updates and final discussions on completed projects including a study of anti Mia antisera in the BC perinatal testing lab as well as an update of testing and labeling strategies for platelet products in fetal/neonatal alloimmune thrombocytopenia.