Canadian Blood Services
Société canadienne du sang

DIAGNOSTIC SERVICES

ALBERTA

YEAR IN REVIEW

JANUARY – DECEMBER 2016

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
- Antibody Titre,
- Phenotyping
- Fetal Bleed Screening Test
- Kleihauer-Betke Test for Quantitation of fetal-maternal hemorrhage
- Postnatal Testing

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 monthly in the first and second trimester and every two weeks in the last trimester. More frequent testing may be indicated if the antibody titre rises rapidly or if clinical monitoring mandates that additional sampling would provide helpful information.

Mothers – Postnatal: Following delivery, specimens from the mother and her baby should be tested if the Rh of the mother is unknown, the mother is Rh negative, the mother has a clinically significant antibody or if the baby shows signs of HDFN (i.e. anemia or jaundice). Midwives or hospitals that do not perform transfusion medicine testing should submit specimens to Canadian Blood Services. A fetal bleed screening test is performed if an Rh negative woman delivers an Rh positive baby. The Kleihauer-Betke assay is performed when the mother has a positive fetal bleed screening test.

Newborns (Cords): Cord blood or neonatal specimens must be submitted with the mother's specimen as noted above. ABO/Rh and Direct Antiglobulin testing is performed on cord or neonatal specimens submitted to Canadian Blood Services

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.

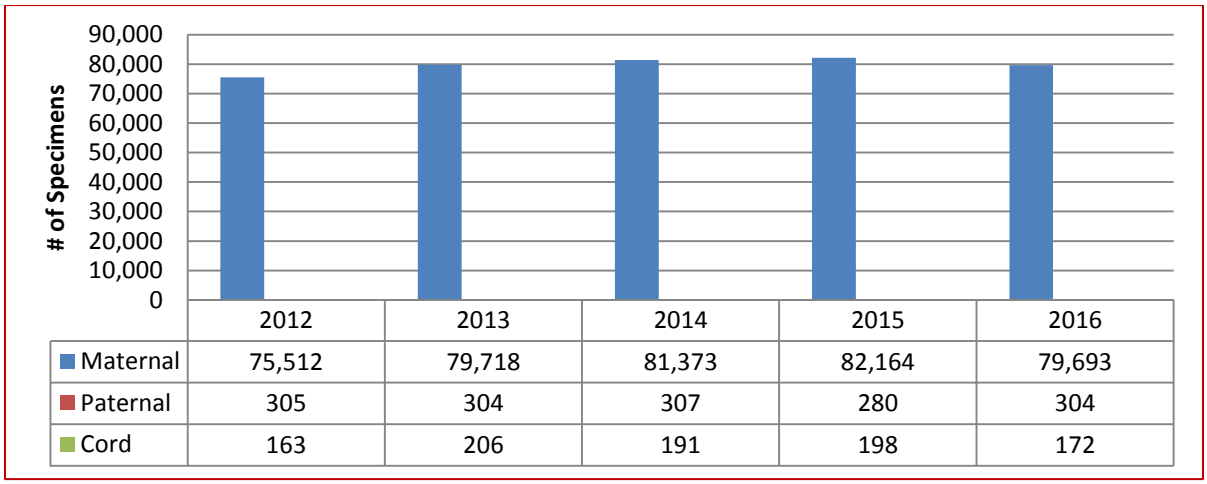
C. Specimens Tested

The data includes all women tested.

Table 1: Perinatal Specimens Tested

Specimen Type	Test Type	2012	2013	2014	2015	2016
Maternal	Type and Screen	75,512	79,718	81,373	82,164	79,693
Paternal	ABO/Rh	305	304	307	280	304
Cord	ABO/Rh	163	206	191	198	172
Total # of Specimens Tested		**75,980**	**80,228**	**81,871**	**82,642**	80,169
Total # of Patients Tested		**63,277**	**68,877**	**67,618**	**68,657**	**66,287**

Figure 1: Total Perinatal Specimens Tested



D. Antibodies Identified

In 2016, a total of 360 antibodies were reported (see *Table 2*). This is lower than 2015. Three hundred and nineteen (319) women had antibodies identified during their pregnancies, and of these, forty-six (46) 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-D, anti-K, anti-c and anti-M which together represented 80% of the total antibodies identified. Titres for 23 of the clinically significant antibodies increased from non-critical to critical levels during the pregnancy with a total of 44 antibody titres at critical levels (see *Table 3*). Recommendations were made for all patients with a critical titre level (current or previous pregnancy including all Kell system antibodies) to be referred to a Maternal-Fetal Medicine Clinic for further follow-up and monitoring during pregnancy.

Table 2: Total Number of Perinatal Antibodies Detected

Maternal Antibodies Identified (Including Passive D) – 2016					
Clinically Significant Antibodies - Antibody	**2012**	**2013**	**2014**	**2015**	**2016**
Anti-D	48	66	67	49	58
Passive Anti-D	702	791	1,119	633	497
Anti-C	0	30	31	17	13
Anti-Cw	1	3	1	2	1
Anti-Ce	0	0	0	0	
Anti-c	45	70	59	41	43
Anti-E	80	134	117	91	106
Anti-e	10	15	15	13	6
Anti-f	0	0	0	0	
Anti-G	0	3	2	3	1
Anti-K	50	77	65	46	53
Anti-S	10	17	16	14	
Anti-s	0	1	1	3	
Anti-U	0	0	1	1	
Anti-Fya	9	10	11	18	12
Anti-Fyb	1	0	3	2	
Anti-Jka	28	41	50	34	18
Anti-Jkb	5	10	6	4	
Anti-JK3	0	0	1	1	
Anti-Lua	3	3	2	0	2
Anti-Lub	3	4	1	1	1
Anti-V	0	1	1	0	
Anti-Vw	0	1	0	0	
Anti-Dia	0	0	1	1	
Anti-Kpa	0	1	1	1	
Anti-Wra	0	11	9	2	5
Total	**995**	**1289**	**1580**	**977**	**816**

Clinically Insignificant Antibodies - Antibody	2012	2013	2014	2015	2016
Anti-A1	6	3	9	8	1
Anti-Lea	12	15	20	6	8
Anti-Leb	1	6	1	1	1
Anti-M	33	40	44	37	29
Anti-N	0	0	2	1	1
Anti-P1	1	0	0	3	
Anti-VS					1
Total	**53**	**64**	**76**	**56**	**41**

Table 3: Perinatal Patient Antibody Titres

Antibody	Critical Level	Non-Critical Level	Non-Critical to Critical Level
Anti-D	15	34	9
Anti-C	1	9	-
Anti-E	13	81	5
Anti-c	5	28	4
Anti-e	-	3	-
Anti-DE	1	-	-
Anti-Ec	4	12	2
Anti-Ce	1	2	1
Anti-G	-	1	-
Anti-Fya	3	7	2
Anti-Jka	-	17	-
Anti-M	-	26	-
Anti-S	1	5	-
Anti-s	-	1	-
Total	**44**	**226**	**23**

Figure 2: Total Number of Perinatal Antibodies

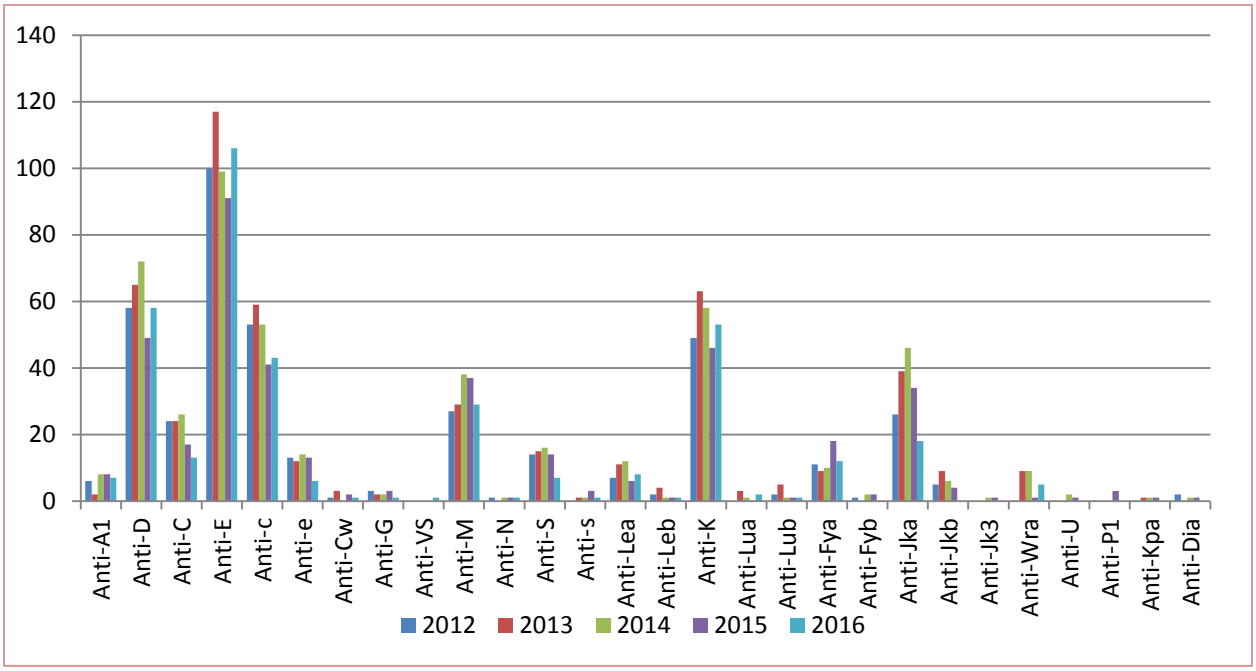


Figure 3: Frequency of Clinically Significant Antibodies

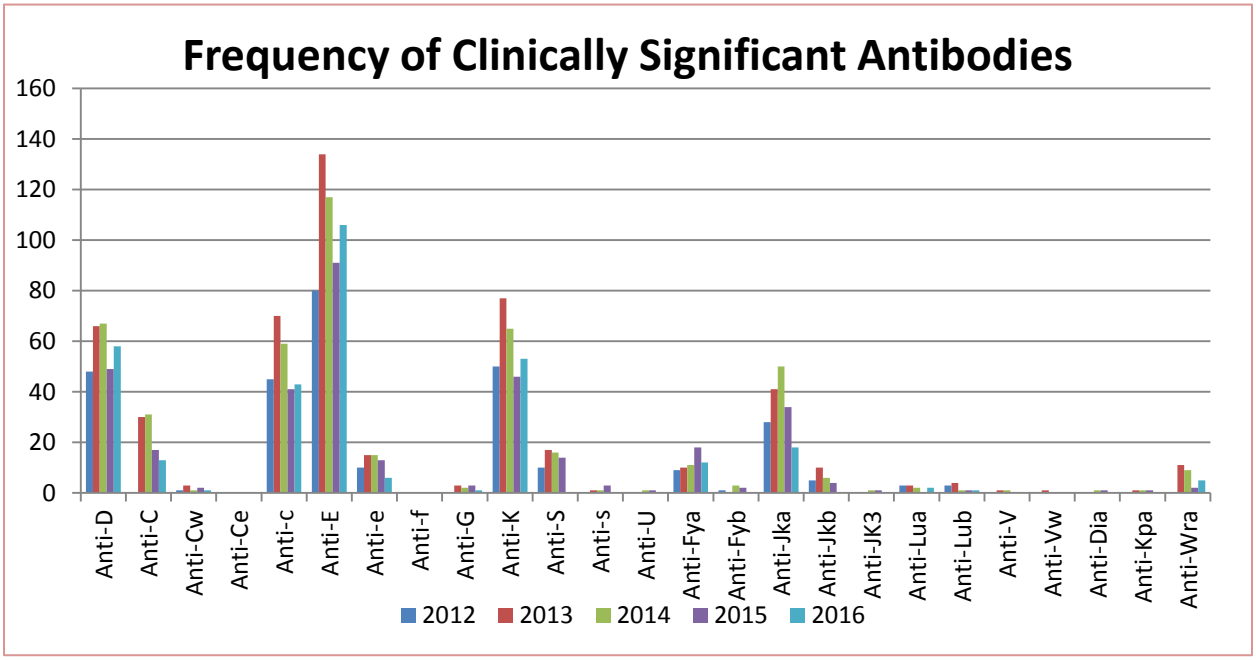


Table 4: Combination Antibodies

Antibodies	Number in 2016
Anti-c, Anti-Cw, Anti-K	1
Anti-C, Anti-D	6
Anti-C, Anti-D, Anti-E	1
Anti-c, Anti-E	13
Anti-C, Anti-e	2
Anti-c, Anti-E, Anti-Jka	1
Anti-C, Anti-G	1
Anti-c, Anti-N	**1**
Anti-D, Anti-Wra	**1**
Anti-E, Anti-Jka	**5**
Anti-E, Anti-Jka, Anti-K	**1**
Anti-E, Anti-K	**2**
Anti-E, Anti-Lea	**1**
Anti-E, Anti-Lua	**1**
Anti-E, Anti-S	**2**
Anti-E, Anti-Wra	**1**
Anti-Fya, Anti-Jka	**2**
Anti-Jka, Anti-K	**1**
Anti-Jka, Anti-Wra	**1**
Anti-K, Anti-Lea	**1**
Anti-Lua, Anti-M	**1**

CROSSMATCH / REFERENCE LABORATORY

The Crossmatch/Reference Laboratory within Diagnostic Services provides transfusion medicine services (Crossmatch) for 24 hospitals in central / northern Alberta, 2 in the Northwest Territories and 1 in Nunavut 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.

A. 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
- Isohemagglutinin titre
- Phenotyping (patient and donor units)

- Transfusion Reaction Investigation
- Direct Antiglobulin Test
- Elution and Adsorption
- Cold Agglutinin Screen

Antibody Screening is routinely performed by solid phase testing. Combinations of solid phase testing and indirect antiglobulin tube testing using PEG for enhancement are the primary antibody identification methods. PEG IAT is also the manual back-up method for antibody screening.

The Crossmatch Laboratory distributes both stock and crossmatched red cell and platelet components to those hospitals which receive all of their transfusion medicine services from Canadian Blood Services. As a Reference Laboratory, the Laboratory performs complex antibody investigations.

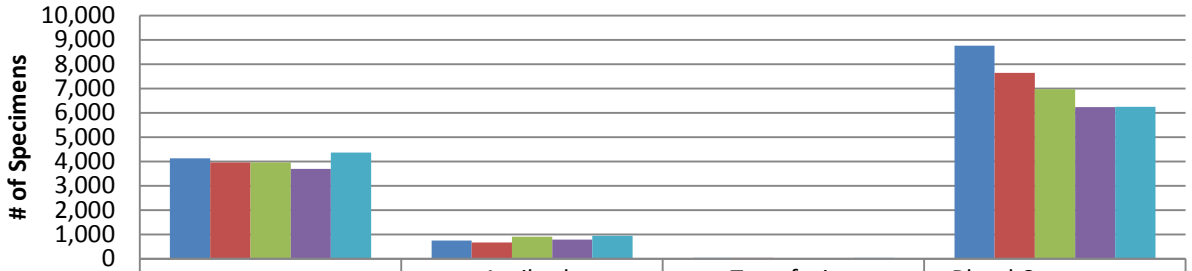
B. **Specimens Tested**

The data in this report reflects a calendar year period to enable better correlation to other government statistical data. The total number of specimens tested has remained relatively stable with fluctuations within 18% year over year as illustrated in *Table 5* below. There has been a steady decrease in the number of components distributed since 2012 but remained relatively the same.

Table 5: Crossmatch/Reference Specimens Tested

Specimen Type	Test Type	2012	2013	2014	2015	2016
Crossmatch/Reference	Type and Screen	4,120	3,950	3,959	3,694	4,360
	Antibody Investigations	741	664	903	787	945
	Transfusion Reaction Investigations	39	35	26	25	32
	Blood Components Distributed	8,752	7,644	6,972	6,228	6,245
Test Totals (excluding components distributed)		**4,900**	**4,649**	**4,888**	**4,506**	**5,337**
Number of Patients Tested		**2,437**	**2,349**	**2,345**	**2,115**	**2,092**

Figure 4: Total Crossmatch Specimens Tested



	Type and Screen	Antibody Investigations	Transfusion Reactions	Blood Components Distributed
2012	4,120	741	39	8,752
2013	3,950	664	35	7,644
2014	3,959	903	26	6,972
2015	3,694	787	25	6,228
2016	4,360	945	32	6,245

C. Antibodies Identified

In 2016, a total of 241 antibodies were reported (see *Table 6*). The total number of antibodies detected is 10.5% higher than in 2015, but the distribution of the most common antibodies remains consistent. One hundred and eighty-eight (188) patients had antibodies identified, and of these, forty (40) patients had multiple antibodies.

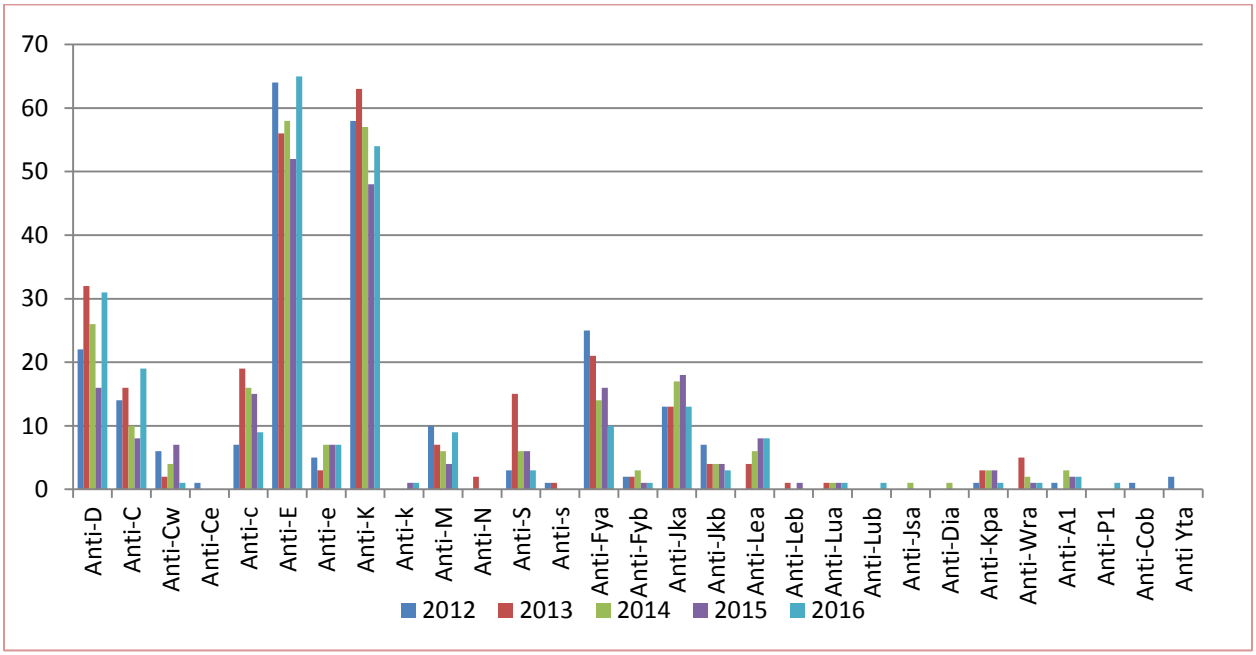
Antibodies identified were considered to be clinically significant if they have been reported to cause acute or delayed hemolytic transfusion reactions. The most common clinically significant antibodies identified were anti-D, anti-C, anti-E, anti-K and anti-Jk[a] (see *Figure 5*) which together represented 75.5% of the total antibodies identified.

Table 6: Total Number of Crossmatch Antibodies Detected

Antibody	2012	2013	2014	2015	2016
Anti-D	22	32	26	16	31
Anti-C	14	16	10	8	19
Anti-Cw	6	2	4	7	1
Anti-Ce	1	0	0	0	0
Anti-c	7	19	16	15	9
Anti-E	64	56	58	52	65
Anti-e	5	3	7	7	7
Anti-K	58	63	57	48	54
Anti-k	0	0	0	1	1
Anti-M	10	7	6	4	9

Anti-N	0	2	0	0	0
Anti-S	3	15	6	6	3
Anti-s	1	1	0	0	0
Anti-Fya	25	21	14	16	10
Anti-Fyb	2	2	3	1	1
Anti-Jka	13	13	17	18	13
Anti-Jkb	7	4	4	4	3
Anti-Lea	0	4	6	8	8
Anti-Leb	0	1	0	1	0
Anti-Lua	0	1	1	1	1
Anti-Lub					1
Anti-Jsa	0	0	1	0	0
Anti-Dia	0	0	1	0	0
Anti-Kpa	1	3	3	3	1
Anti-Wra	0	5	2	1	1
Anti-A1	1	0	3	2	2
Anti-P1					1
Anti-Cob	1	0	0	0	0
Anti Yta	2	0	0	0	0
Total	**243**	**270**	**245**	**219**	**241**

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. Specimens are also referred 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.

A total of 23 maternal samples from 22 patients were sent for cffDNA analysis in 2016 compared to twenty (20) samples from seventeen (17) patients in 2015 (see *Table 7*).

Table 7: Fetal Genotyping Results Summary

	2013	2014	2015	2016
Total samples sent	18	24	20	23
# patients tested	15	18	17	22
# patients not requiring MFM follow-up (Tested negative for the corresponding antigen)	6	10	4	9

Table 8: Fetal Phenotyping Results Summary

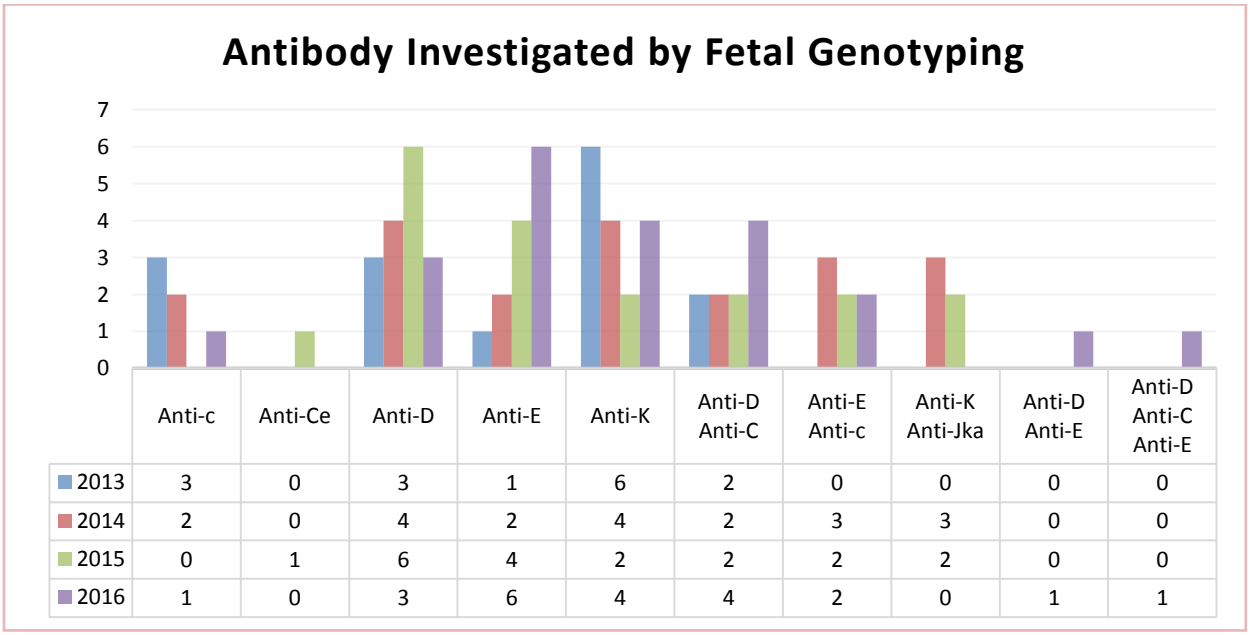
Patient	Maternal Antibody	Predicted Fetal Phenotype	Follow-up required?
1	Anti-E, Anti-c	Unable to type	Yes
2	Anti-D	RhD Pos	Yes
3	Anti-D, Anti-C	RhD Pos, RhC NT	Yes*
4	Anti-D, Anti-C	RhD Neg, RhC NSQ	No**
5	Anti-E	RhE Neg	No

6	Anti-K	K Pos	Yes
7	Anti-E	RhE Pos	Yes
8	Anti-D, Anti-C, Anti-E	Unable to type	Yes
9	Anti-D, Anti-C	RhD Pos, RhC NT	Yes*
10	Anti-E	RhE Neg	No
11	Anti-D	RhD Pos	Yes
12	Anti-D	RhD Neg	No
13	Anti-D, Anti-E	RhD Pos, RhE Pos	Yes
14	Anti-D, Anti-C	RhD Neg, RhC NT	No**
15	Anti-E	RhE Neg	No
16	Anti-K	K Neg	No
17	Anti-E, Anti-c	RhE Pos, Rhc unable to type	Yes
18	Anti-K	K Neg	No
19	Anti-E	RhE Pos	Yes
20	Anti-E	RhE Pos	Yes
21	Anti-K	Inconclusive	Yes
22	Anti-c	Rhc Neg	No

*MFM monitoring required for Anti-D.
**Other antibody is either not detected or titre is <1.

In 2016, the most commonly probed antibodies were anti-D and Anti-E and anti-K (see *Figure 6*). The number of samples sent for RHD genotyping has increased slightly since 2015.

Figure 6: Total Number of Antibodies Probed by Fetal Genotyping



	Anti-c	Anti-Ce	Anti-D	Anti-E	Anti-K	Anti-D Anti-C	Anti-E Anti-c	Anti-K Anti-Jka	Anti-D Anti-E	Anti-D Anti-C Anti-E
2013	3	0	3	1	6	2	0	0	0	0
2014	2	0	4	2	4	2	3	3	0	0
2015	0	1	6	4	2	2	2	2	0	0
2016	1	0	3	6	4	4	2	0	1	1

RHD RED CELL GENOTYPING

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 2016, the following RHD Genotyping results were obtained:

Figure 7: Rh D Testing Algorithm

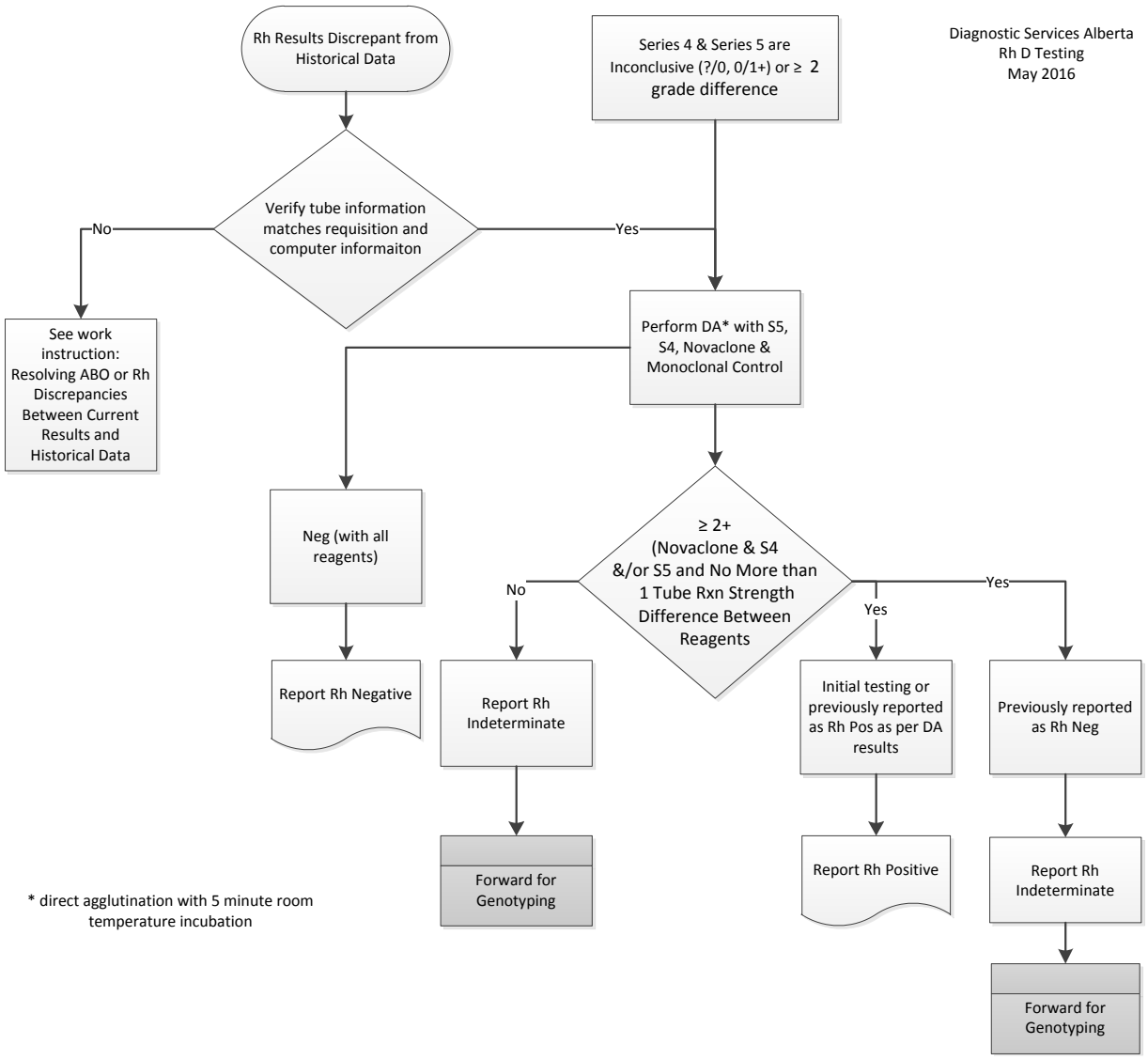


Figure 8: Number of RHD Genotyping Alleles Detected

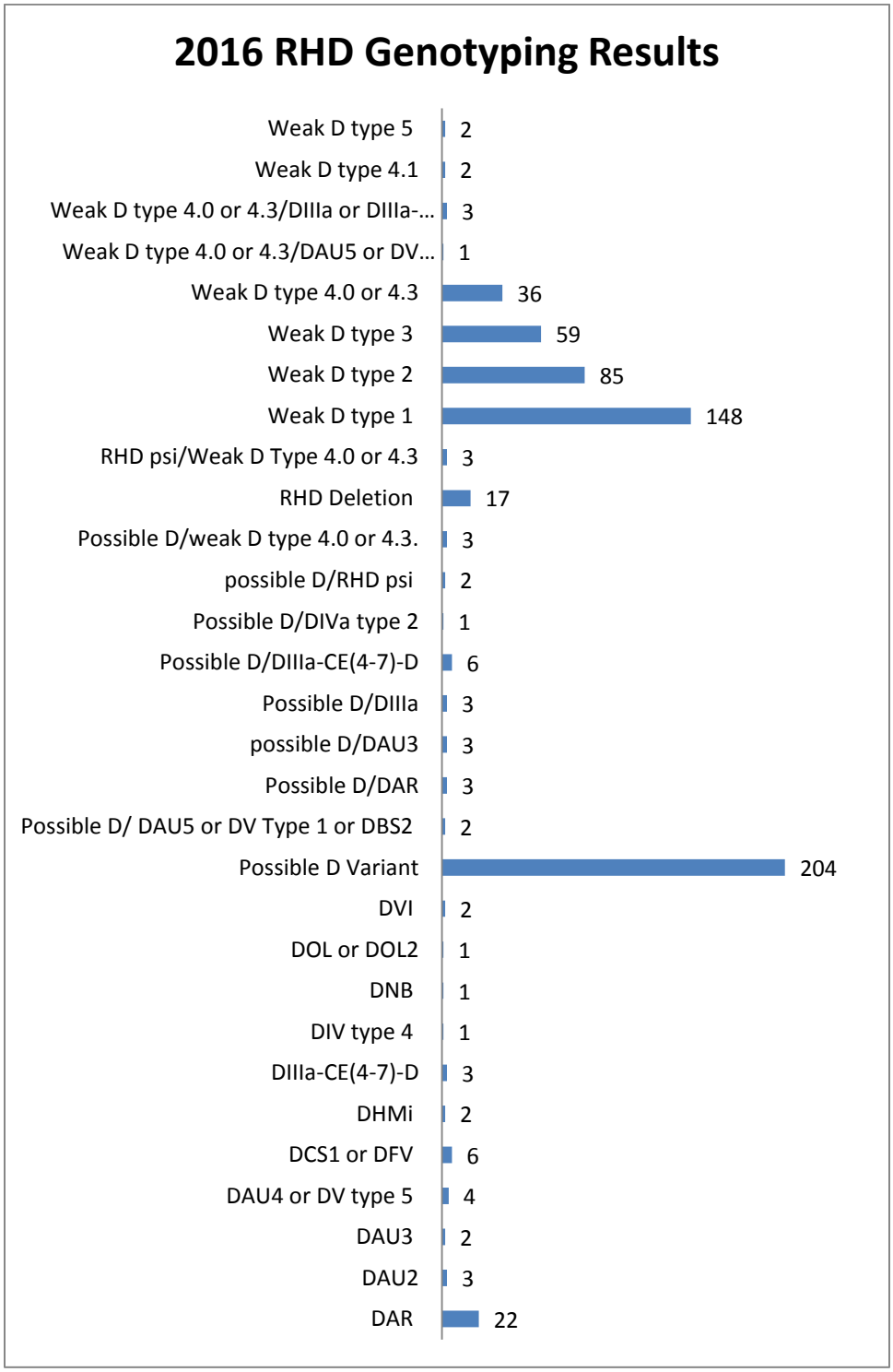
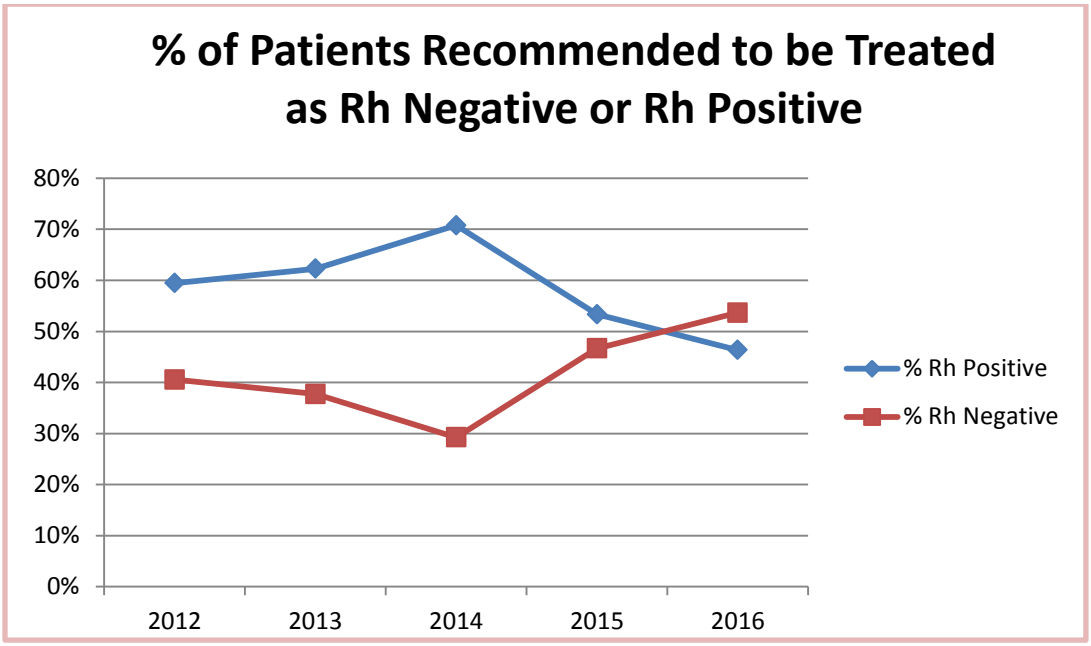


Table 9: RHD Genotyping – Number of Rh Negative and Rh Positive Predicted Phenotypes

	2012	2013	2014	2015	2016
Rh Positive	22	33	92	200	292
Rh Negative	15	20	38	175	338
Total # samples tested	**37**	**53**	**130**	**375**	**630**

Figure 9: RHD Genotyping - % Patients Recommended to be Treated as Rh Negative and Rh 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 specimens are received at Canadian Blood Services in Edmonton to the time when the results are available. Since monitoring of this quality indicator began in 2008, the percentage of perinatal specimens has been close to the predefined TAT threshold. The percentage of crossmatch/reference specimens has consistently met the predefined TAT threshold. Samples whose testing exceeded the expected TAT are usually those where clinically significant antibodies are detected or where difficulty in finding compatible blood is encountered.

Table 10: Turnaround Time – Routine Criteria by Specimen Type

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

Figure 10: Perinatal Routine TAT

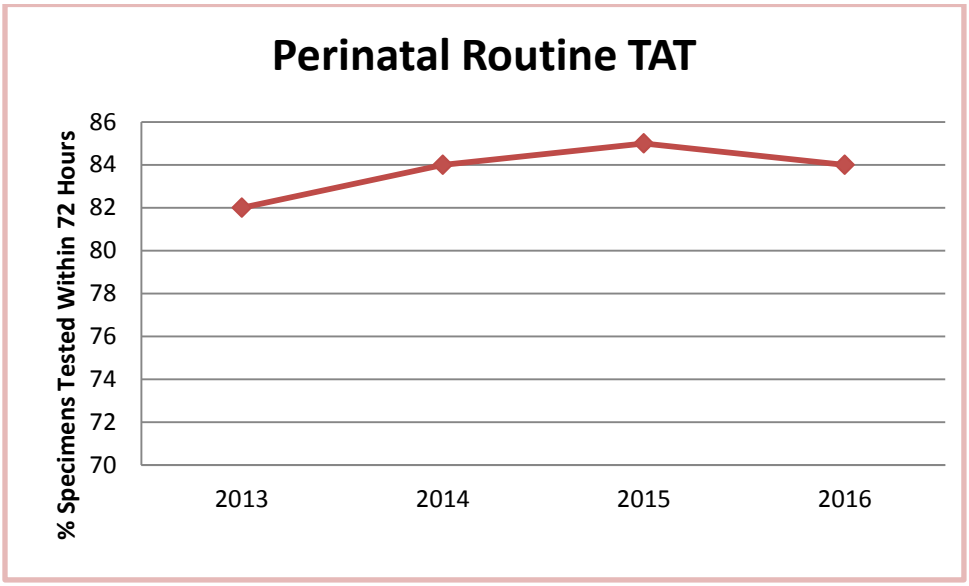


Table 11: Turnaround Time – Routine Perinatal Specimens

Turnaround Time	2013	2014	2015	2016
% of Specimens Tested within 72 hours	82%	84%	85%	84%
% of Specimens Tested > 72 hours	17%	16%	15%	16%

Figure 11: Crossmatch Routine TAT

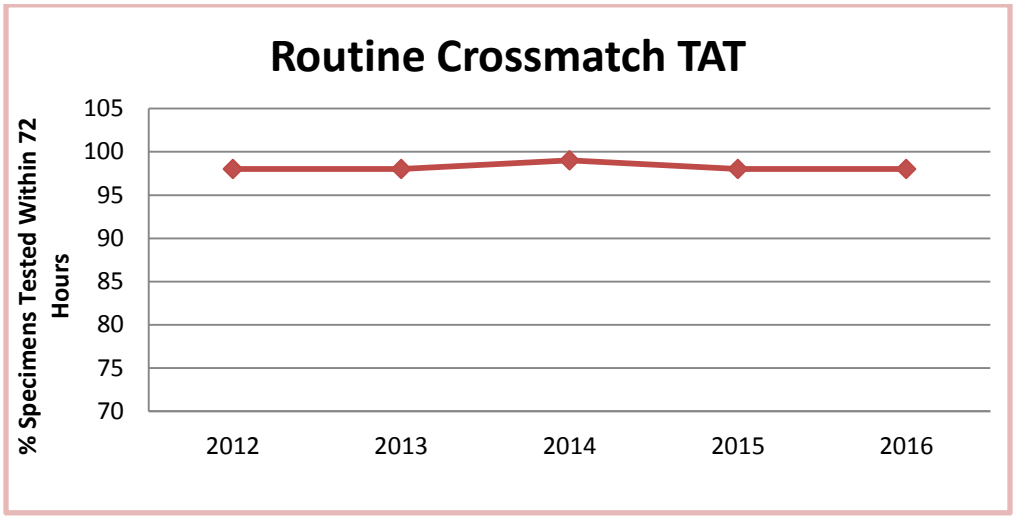


Table 12: Turnaround Time – Routine Crossmatch Specimens

Turnaround Time	2012	2103	2014	2015	2016
% of Specimens Tested within 24 hours	99%	98%	99%	99%	99%
% of Specimens Tested > 24 hours	1%	3%	2%	1%	1%

Table 13: Reference TAT

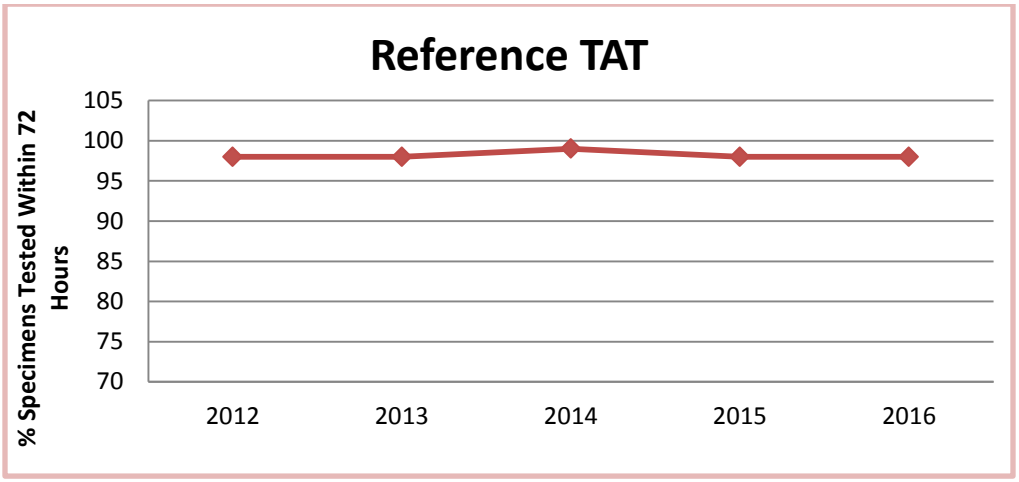


Figure 12: Turnaround Time – Reference Specimens

Turnaround Time	2012	2013	2014	2015	2016
% of Specimens Tested within 72 hours	98%	98%	99%	98%	98%
% of Specimens Tested > 72 hours	4%	2%	2%	2%	2%

B. 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.

For perinatal specimens, the most common reasons for rejecting a sample for testing are patient identification labelling errors and duplicate requests for testing (duplicate specimens). Testing requests are rejected if another sample from the patient was tested within the previous 96 hours. Often a duplicate test request 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.

Table 14: Quarterly Rejection Rates – Perinatal Specimens

Rejection Category	Q1	Q2	Q3	Q4
Requisition	26	17	22	19
Specimen	19	39	40	31
Discrepancies Between Requisition & Specimen	20	7	6	7
Discrepancies Between Current Requisition & Historical Records	0	0	0	0
Other (Duplicates, etc.)	25	12	12	16
Total # specimens rejected	90	75	80	73
Total # specimens received	19503	18959	19323	20005
Rejections as a % of total	**.04%**	**.04%**	**.04%**	**.04%**

Figure 13: Perinatal Rejection Reasons

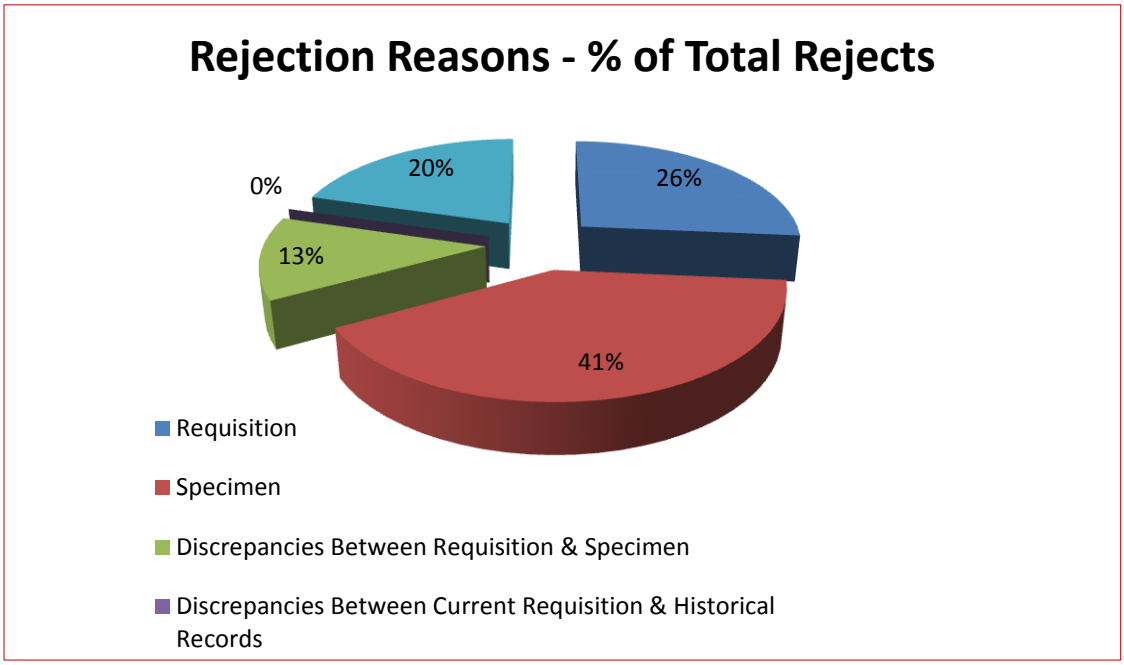
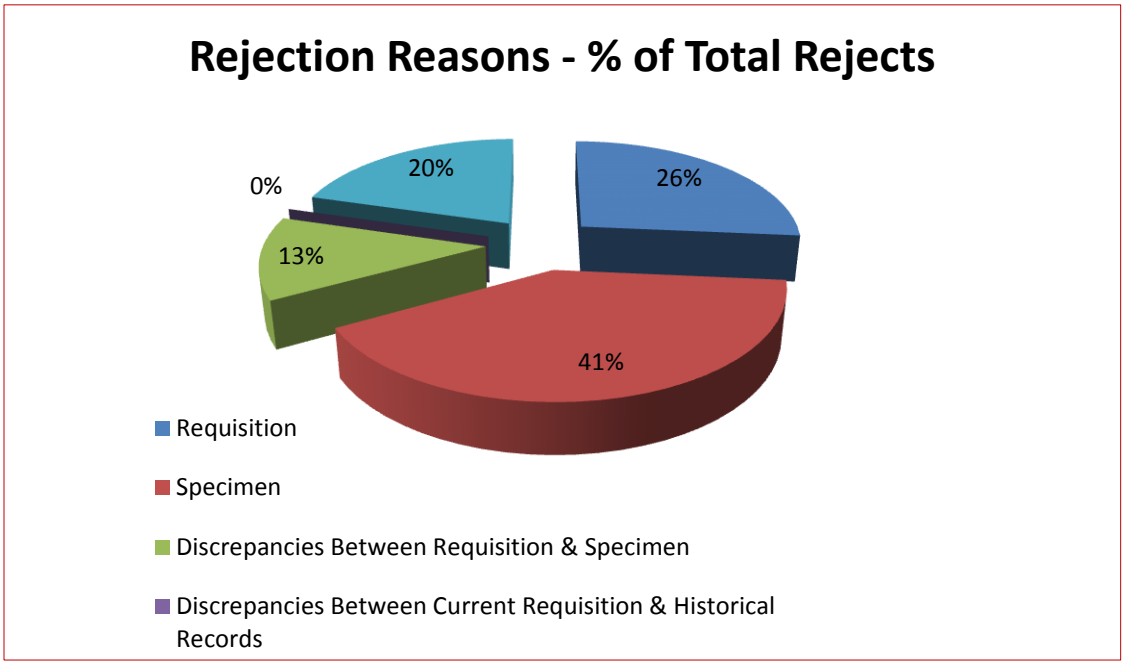


Table 15: Quarterly Rejection Rates – Crossmatch Specimens

Rejection Category	Q1	Q2	Q3	Q4
Requisition	1	0	3	2
Specimen	1	3	0	1
Discrepancies Between Requisition & Specimen	0	0	1	0
Discrepancies Between Current Requisition & Historical Records	0	0	0	0
Other (Duplicates, etc.)	7	0	4	6
Total # specimens rejected	9	3	8	9
Total # specimens received	893	1075	1173	1265
Rejections as a % of total	**1%**	**.02%**	**.06%**	**.07%**

Figure 14: Crossmatch Rejection Reasons



ACCOMPLISHMENTS IN 2016

A. RHD Genotyping

Implementation of the licensed RHD testing platform in January 2016. Post implementation reviews performed to continue to improve and refine the reporting process.

B. Mis-Transfusion Risk Reduction Strategy

Implementation of the mis-transfusion risk reduction strategy in April 2016

C. Perinatal Advisory Committee

The Perinatal Advisory Committee meeting for 2016 was held on June 13th and 14th. This year, the PNAC meeting was hosted in Winnipeg and was held in conjunction with a Grifol's Transfusion Science Education Course.

The PNAC meeting covered a range of topics relevant to the CBS diagnostic and perinatal laboratories. Our agenda included a review of laboratory internal audits which allowed us to compare practices across laboratories and identify areas for improvement and standardization. Specific standardization initiatives incluing the antibody investigation algorithm for prenatal patients, the strategy and algorithm used for assessment of serological weak D patients through genotyping, and recommendations related to standard timing for prenatal sample testing were discussed. We developed a strategy for investigation of anti G in prenatal patients and discussed the feasibility of enhanced automated testing.

Results of projects from the prior year were also reviewed. These included the results of an audit amongst hospital transfusion services regarding the feasibility of using Kell negative phenotyped red cell units for transfusion to female patients of child bearing potential, as well as the results of a study into the utility of a new monoclonal anti Mia antibody.

In follow up to the 2016 meeting several projects have been selected for additional work. These include continued work on alignment of the algorithm for assessment for weak and partial D antigens by RHD genotyping, additional work on the development and standardization of automated testing for passive anti-D evaluation, and agreement on timing of sample testing for perinatal patients.

GOALS FOR 2017

A. Restructure Laboratory Services

Participate with AHW and AHS in discussions related to the repatriation of crossmatch services with a focus on a smooth transition with minimal impact to patient care.

Participate in discussions related to the National Facilities Redevelopment Plan Phase II for testing services that are to remain in Edmonton.

B. Testing Standardization

Support initiatives to standardize work instructions across Canadian Blood Services Testing laboratories.