DIAGNOSTIC SERVICES

ALBERTA

YEAR IN REVIEW

JANUARY – DECEMBER 2022

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
- Antibody Titration
- 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, maternal trauma, or obstetrical procedure).

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. Less frequent sampling may also be recommended for antibodies that are unlikely to be clinically significant or in cases where clinical monitoring through fetal Doppler ultrasound has commenced.

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. The direct antiglobulin test is performed if the mother has a clinically significant

antibody or on request if the baby shows signs of HDFN (i.e., anemia or jaundice). This is especially important when the mother is Rh negative or when the mother has a clinically significant antibody. If the baby has unexpected anemia or jaundice assessment of the cord blood sample for blood group and DAT may also be helpful.

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.

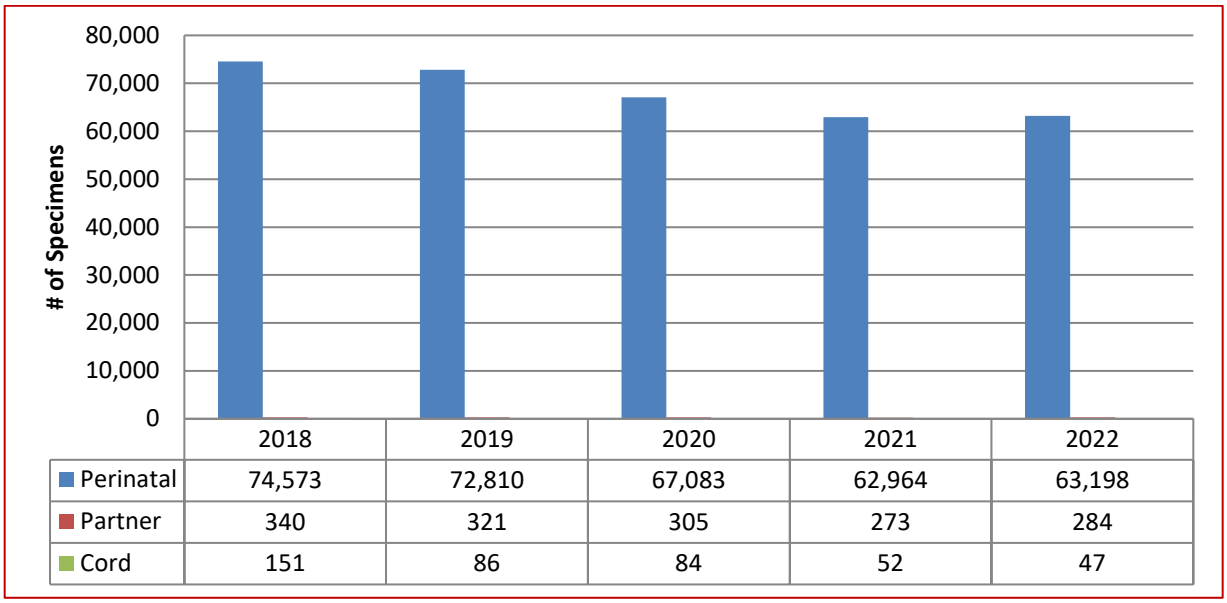
C. Specimens Tested

The data includes all women tested.

Table 1: Perinatal Specimens Tested

Specimen Type	Test Type	2018	2019	2020	2021	2022
Maternal	Type and Screen	74,573	72,810	67,083	62,964	63,198
Paternal	ABO/Rh	340	321	305	273	284
Cord	ABO/Rh	151	86	84	52	47
Total # of Specimens Tested			**75,064**	**73,217**	**67,472**	**63,289**
Total # of Patients Tested		**Not reported**	**Not reported**	**58,769**	**55,091**	**54,462**

Figure 1: Total Perinatal Specimens Tested



D. Antibodies Identified

In 2022, a total of 561 antibodies were reported (see *Table 2*). This is higher than 2021 where 424 antibodies were reported. Of 561 antibodies identified in 2022, 387 women had clinically significant antibodies, 74 had clinically insignificant antibodies and 63 women had multiple antibodies. Passive anti-D data has been excluded from the preceding numbers.

Antibodies identified are considered to be clinically significant if they have been reported to cause HDFN. The most common clinically significant antibodies identified were: anti-E, anti-c, anti-D, anti-K, anti-Jka, (see *Figure 2*) which together represented 72% of the total antibodies identified. IgG Anti-M can also be considered clinically significant as it may cause HDFN and/or delayed neonatal anemia in rare cases.

Titres for 23 of the clinically significant antibodies increased from non-critical to critical levels during the pregnancy with a total of 58 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					
Clinically Significant Antibodies	**2018**	**2019**	**2020**	**2021**	**2022**
Anti-D	48	54	52	40	51
Anti-C	26	6	11	23	20
Anti-Cw	0	0	0	0	0
Anti-c	65	29	25	44	42
Anti-E	150	135	121	113	112
Anti-e	4	7	9	11	9
Anti-f	0	0	0	0	0
Anti-G	6	2	2	5	5
Anti-K	70	52	43	47	56
Anti-M*	52	38	36	36	28
Anti-S	13	4	8	12	11
Anti-s	0	2	2	3	1
Anti-U	2	1	0	0	0
Anti-Fya	12	4	7	14	18
Anti-Fyb	3	1	0	0	1
Anti-Jka	28	20	30	38	39
Anti-Jkb	4	3	3	4	3
Anti-JK3	0	0	1	0	0
Anti-Lua	2	1	0	1	0
Anti-Lub	2	1		2	
Anti-Dia	0	1	1	0	0
Anti-Kpa	2	0	0	0	0
Anti-Wra	5	1	2	4	4

Maternal Antibodies Identified					
Clinically Significant Antibodies	**2018**	**2019**	**2020**	**2021**	**2022**
Anti-Jsa	0	0	0	0	0
Anti-Mia	1	1	0	1	0
Anti-Joa	1	0	0	2	0
Anti-Yta	2	1	0	0	0
Anti-Mur	1	0	0	0	0
Anti-PP1Pk	1	0	0	1	0
Anti-Sc2	0	1	0	0	0
Anti-Cob	0	0	1	0	0
Anti-Dia	0	0	0	3	1
Anti-Vel	0	0	0	1	0
Anti-Hr	0	0	0	1	0
Panreactive Autoantibody	0	0	16	17	16
Antibody to a Low Prevalence Antigen	0	0	1	1	0
Total	**500**	**364**	**353**	**401**	**417**

***Anti-M – IgG antibody detected**

Maternal Antibodies Identified					
Clinically Insignificant Antibodies	**2018**	**2019**	**2020**	**2021**	**2022**
Anti-A1		10	2	8	11
Anti-Lea	20	11	15	16	9
Anti-Leb	3	3	2	1	0
Anti-N	1	2	1	1	1
Anti-P1	2	1	0	1	1
Anti-VS	0	0	0	0	0
Anti-Ytb	0	0	0	1	0
Cold agglutinin	0	0	9	8	16
Panreactive Antibody	0	0	0	0	6
Warm Autoantibody	0	0	0	0	1
Unidentified Antibody	Not reported	Not reported	21	23	43

Maternal Antibodies Identified					
Clinically Insignificant Antibodies	**2018**	**2019**	**2020**	**2021**	**2022**
Antibody of Undetermined Significance	Not reported	Not reported	Not reported	Not reported	56
Passive Anti-D *(not included in totals)*	555	855	726	601	453
TOTAL: Clinically Insignificant Antibodies	**26**	**17**	**18**	**19**	**144**

Table 3: Perinatal Patient Antibody Titres 2022

Antibody	Critical Level	Non-Critical Level	Non-Critical to Critical
Anti-A1	1		
Anti-C		11	
Anti-c	5	29	3
Anti-Ce	1	2	
Anti-CG	1	3	
Anti-Cw			
Anti-D	13	40	6
Anti-DC	2		
Anti-DE	1	2	1
Anti-DG	1	1	1
Anti-Dia		1	
Anti-E	16	87	
Anti-e	2	7	5
Anti-Ec	5	11	2
Anti-Fya	5	14	1
Anti-Fyb		1	
Anti-G		1	
Anti-K		1	
Anti-Jka	2	40	2
Anti-Jkb	1	3	1
Anti-M	1	27	1
Anti-S	1	10	
Anti-s		1	
Anti-Wra		4	

Figure 2: Total Number of Perinatal Antibodies

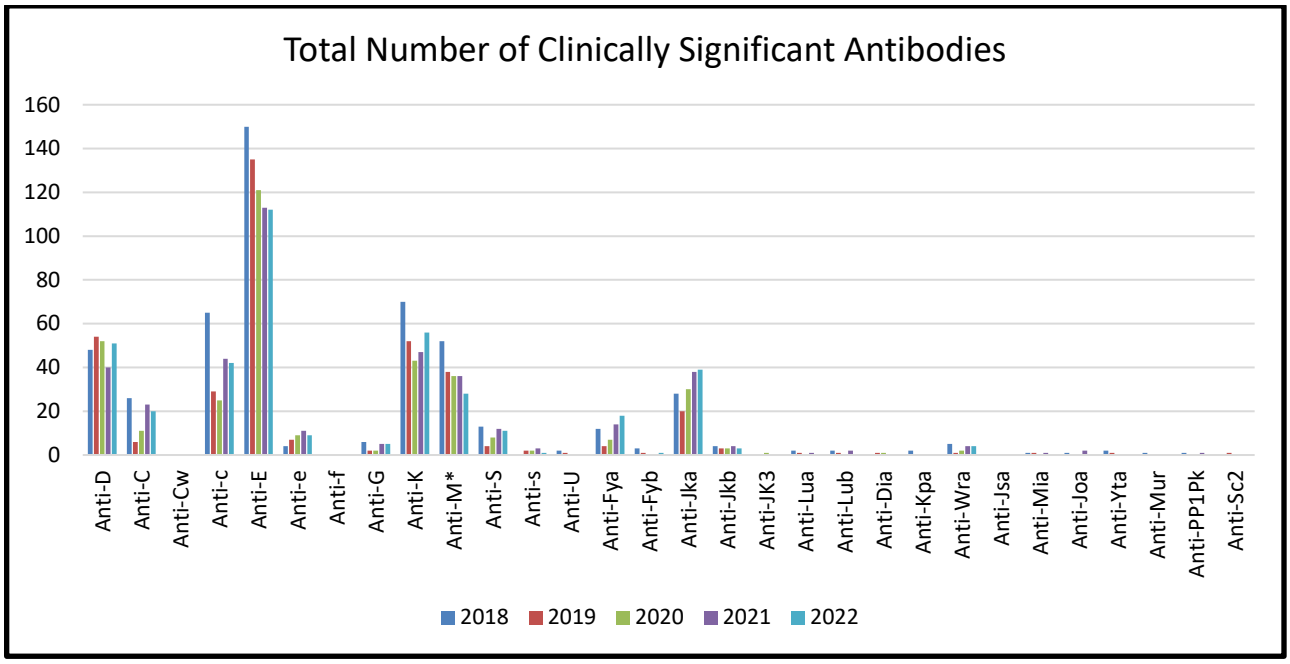


Figure 3: Frequency of Clinically Significant Antibodies

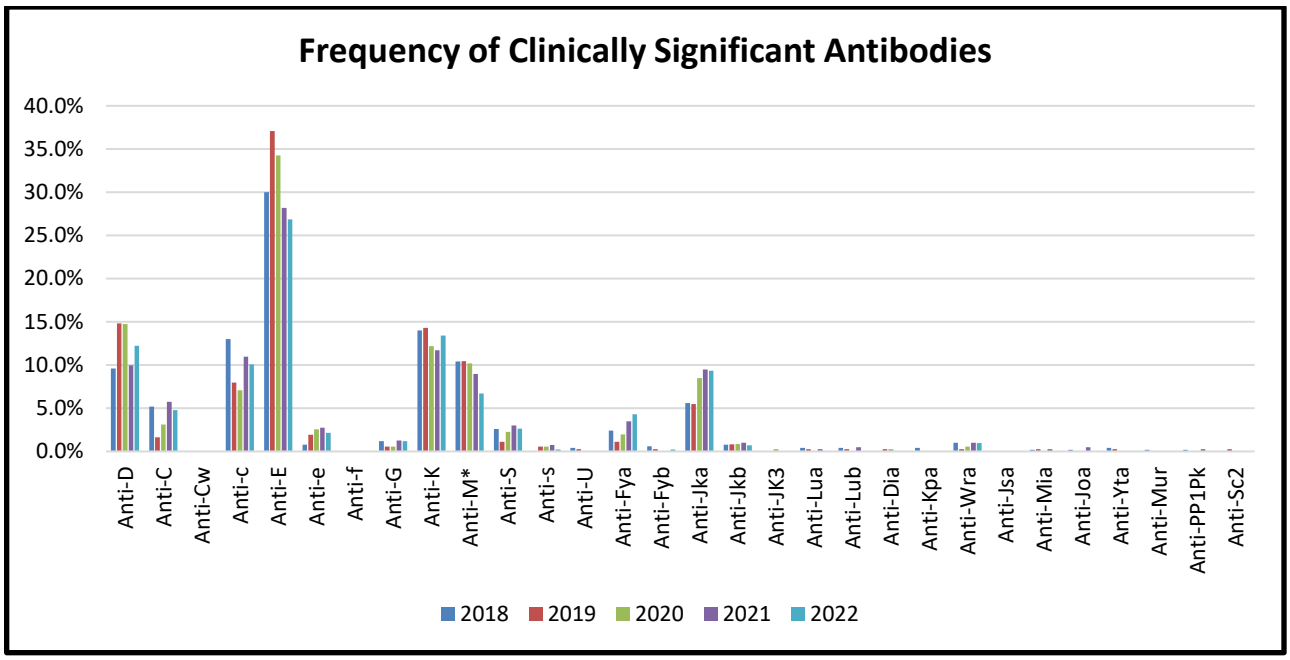


Table 4: Perinatal Combination Antibodies 2022

Combination Antibodies	Prenatal
Anti-C Antibody of Undetermined Significance	1
Anti-C Anti-D	1
Anti-c Anti-E	7
Anti-C Anti-e	1
Anti-c Anti-E Anti-Fya	1
Anti-c Anti-E Anti-Jka	1
Anti-c Anti-E Anti-K	1
Anti-C Anti-e Anti-S	1
Anti-c Anti-E Anti-S Anti-Jka Unidentified Antibody	1
Anti-c Anti-E Unidentified Antibody	2
Anti-C Anti-e Unidentified Antibody	1
Anti-c Anti-Fya Unidentified Antibody	1
Anti-C Anti-G	3
Anti-C Anti-G Anti-S	1
Anti-c Anti-Jka	1
Anti-c Anti-K Anti-Jka	1
Anti-c Anti-M	1
Anti-c Anti-S	1
Anti-C Anti-Wra	1
Anti-C Cold Agglutinin Unidentified Antibody	1
Anti-c Panreactive antibody Unidentified Antibody	1
Anti-c Unidentified Antibody	2
Anti-D Antibody of Undetermined Significance	1
Anti-D Anti-E Anti-Fya Anti-Jka	1
Anti-D Anti-E Unidentified Antibody	1
Anti-D Anti-Fya Anti-Jka	1
Anti-D Anti-G	1
Anti-D Anti-Wra	1
Anti-D Panreactive antibody Panreactive Autoantibody Unidentified Antibody	1
Anti-D Unidentified Antibody	1
Anti-E Anti-Fya	1
Anti-e Anti-Jka	1
Anti-E Anti-Jka	2
Anti-E Anti-K	2
Anti-E Anti-Lea	1

Combination Antibodies	Prenatal
Anti-e Anti-M	1
Anti-E Panreactive Autoantibody Unidentified Antibody	1
Anti-E Unidentified Antibody	2
Anti-E Unidentified Antibody Antibody of Undetermined Significance	1
Anti-Fya Unidentified Antibody	1
Anti-Jka Anti-Lea Unidentified Antibody	1
Anti-Jka Anti-P1 Unidentified Antibody	1
Anti-Jka Unidentified Antibody	2
Anti-K Anti-Jka Unidentified Antibody	1
Anti-Lea Unidentified Antibody	2
Anti-M Unidentified Antibody	1
Anti-S Antibody of Undetermined Significance	1
Anti-S Anti-Fyb	1
Anti-S Anti-Jka	1
Anti-s Unidentified Antibody	2
Cold Agglutinin Antibody of Undetermined Significance	1
Cold Agglutinin Panreactive Antibody	3
Cold Agglutinin Panreactive antibody Antibody of Undetermined Significance	1
Cold Agglutinin Panreactive Autoantibody	4
Cold Agglutinin Panreactive Autoantibody Antibody of Undetermined Significance	1
Cold Agglutinin Panreactive Autoantibody Unidentified Antibody	1
Cold Agglutinin Unidentified Antibody	1
Warm Autoantibody Unidentified Antibody	1

REFERENCE LABORATORY

The Reference Laboratory, Edmonton Diagnostic Services provides testing for hospital transfusion medicine laboratories. Hospital patients who are repeatedly transfused may develop red cell antibodies and as a result may have difficulty in tolerating transfusions. Diagnostic Services has specialized and experienced technologists that assist and provide consultation to hospital transfusion medicine laboratories. The Reference Laboratory identifies red cell antibodies, blood group discrepancy resolution, direct antiglobulin testing, fetal bleed screening and other serological testing.

Staff within our department may collaborate with other references laboratories such as the National Immunohematology Reference laboratory (NIRL).

Diagnostic Services Red Cell Antibody Investigations

In 2022, hospitals have referred 263 requests for red cell antibody identification.

Diagnostic Services provides support to hospitals in Alberta, Northwest Territories, northeastern British Columbia and Lloydminster, Saskatchewan. Referring hospitals have different capabilities and expertise in resolving red cell antibody investigations. Some hospitals have limited reagents for antibody identification or phenotyping of patient or donor units. Others have access to a wider variety of reagent red cell panels and methods as well as on site immunohematology expertise. A few hospital transfusion medicine laboratories have the resources to resolve the majority of serological problems and send only complex investigations for additional serological or genotyping studies.

Canadian Blood Services, Diagnostic Services provides consultation and testing support including antibody investigation, advanced or alternative techniques where required, and recommendations for compatibility testing methods and selection of appropriate donor unit phenotypes if necessary.

Reporting may include interim, final and supplemental reports, depending on the urgency of the testing, the need for patient transfusion and the complexity of the investigation. When a new antibody is identified by the Diagnostic Services Laboratory, a patient wallet card may be provided.

A. Testing Performed

The Reference Laboratory routinely performs the following tests:

- ABO/Rh blood type
- Screen for red blood cell antibodies
- Antibody Identification, if antibodies are detected
- Phenotyping
- 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.

As a Reference Laboratory, the laboratory performs complex antibody investigations.

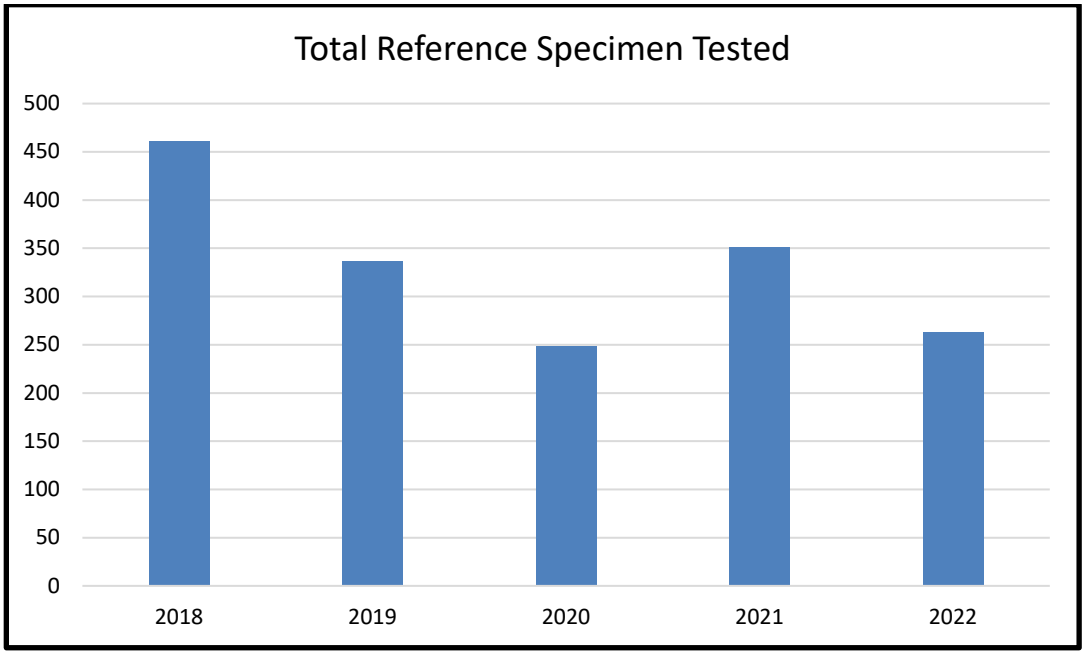
B. **Specimens Tested**

The data in this report reflects a calendar year period to enable better correlation to other government statistical data (Statistics Canada birth statistics).

Table 5: Reference Specimens Tested

Specimen Type	2018	2019	2020	2021	2022
Total Reference Antibody Investigations	461	337	248	351	263

Figure 4: Total Reference Specimens Tested



C. Antibodies Identified

In 2022, a total of 255 antibodies were reported (see *Table 6*). The total number of antibodies detected is higher than in 2021, but the distribution of the most common antibodies remains consistent. One hundred and thirty-three (133) patients had antibodies identified, and of these, forty-one (41) patients had multiple antibodies.

Antibodies identified are 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-E, and anti-K(see *Figure 5*) which together represented 25% of the total antibodies identified.

Table 6: Total Number of Reference Antibodies Detected

Reference Antibodies Identified (Including Passive D) – *Prior to 2019 numbers included Crossmatch samples.					
Antibodies	**2018**	**2019**	**2020**	**2021**	**2022**
Anti-D	14	8	10	6	14
Anti-C	11	4	7	7	6
Anti-Cw	1	2	1	1	2
Anti-c	10	2	6	9	6
Anti-E	52	25	24	14	28
Anti-e	5	4	2	1	3
Anti-G	0	0	1	0	0
Anti-K	47	24	25	8	23
Anti-k	0	0	0	0	0
Anti-Kpa	0	0	1	1	2
Anti-M	8	9	4	0	6
Anti-N	0	0	1	0	0
Anti-S	6	1	4	3	5
Anti-s	0	2	1	0	0
Anti-U	0	0	1	0	0
Anti-Fya	9	3	5	1	9
Anti-Fyb	1	0	1	0	3
Anti-Jka	12	9	10	3	9
Anti-Jkb	2	0	7	3	1
Anti-Lea	2	2	3	2	2
Anti-Leb	0	0	0	1	0
Anti-Lua	0	0	0	0	0
Anti-Lub	0	0	0	0	0
Anti-Fy3	1	0	0	0	0
Anti-Kpa	3	1	0	0	0

Reference Antibodies Identified (Including Passive D) – *Prior to 2019 numbers included Crossmatch samples.					
Antibodies	**2018**	**2019**	**2020**	**2021**	**2022**
Anti-Wra	0	2	4	0	1
Anti-A1	1	0	0	0	0
Anti-P1	1	0	0	0	0
Anti-Cob	0	0	1	0	1
Anti Yta	0	0	0	0	0
Anti-IH	0	1	0	0	0
Anti-JMH	0	1	0	0	0
Anti-Jsa	0	0	0	1	0
Anti-Kpa	0	0	0	1	0
Anti-Ce	0	0	0	0	1
Anti-V	0	0	0	1	0
Panreactive Autoantibody	Not reported	16	22	5	16
Antibody to a Low Prevalence Antigen	0	0	1	0	0
Unidentified Antibody	Not reported	6	19	12	15
Cold Agglutinin	Not reported	20	18	12	24
Autoantibody	0	1	2	4	4
Antibody to an HLA related antigen	0	0	0	0	1
Antibody of Undetermined Significance	Not reported	Not reported	Not reported	Not reported	6
Panreactive Antibody	Not reported	5	12	6	11
Passive Anti-D	Not reported	Not reported	88	67	56
Total	**186**	**148**	**281**	**169**	**255**

Figure 5a: Total Number of Reference Antibodies

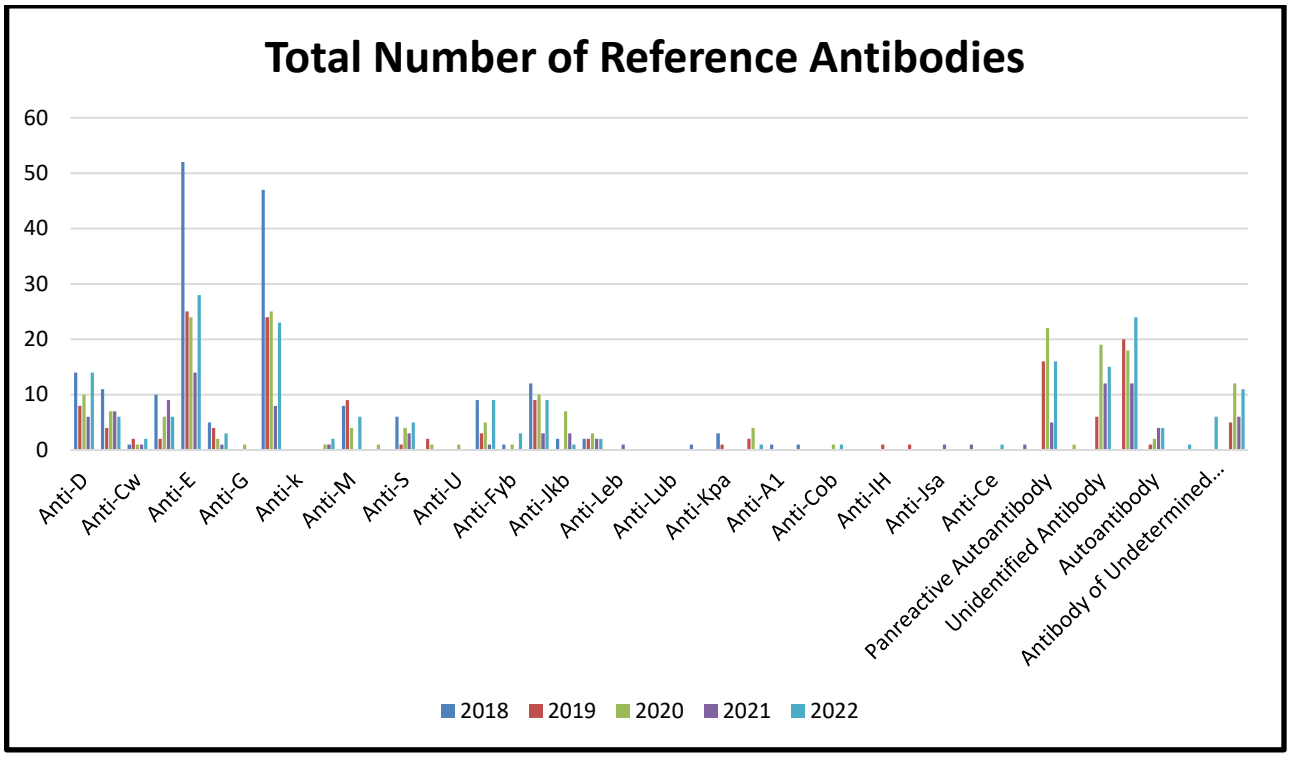


Figure 5b: Frequency of Reference Antibodies

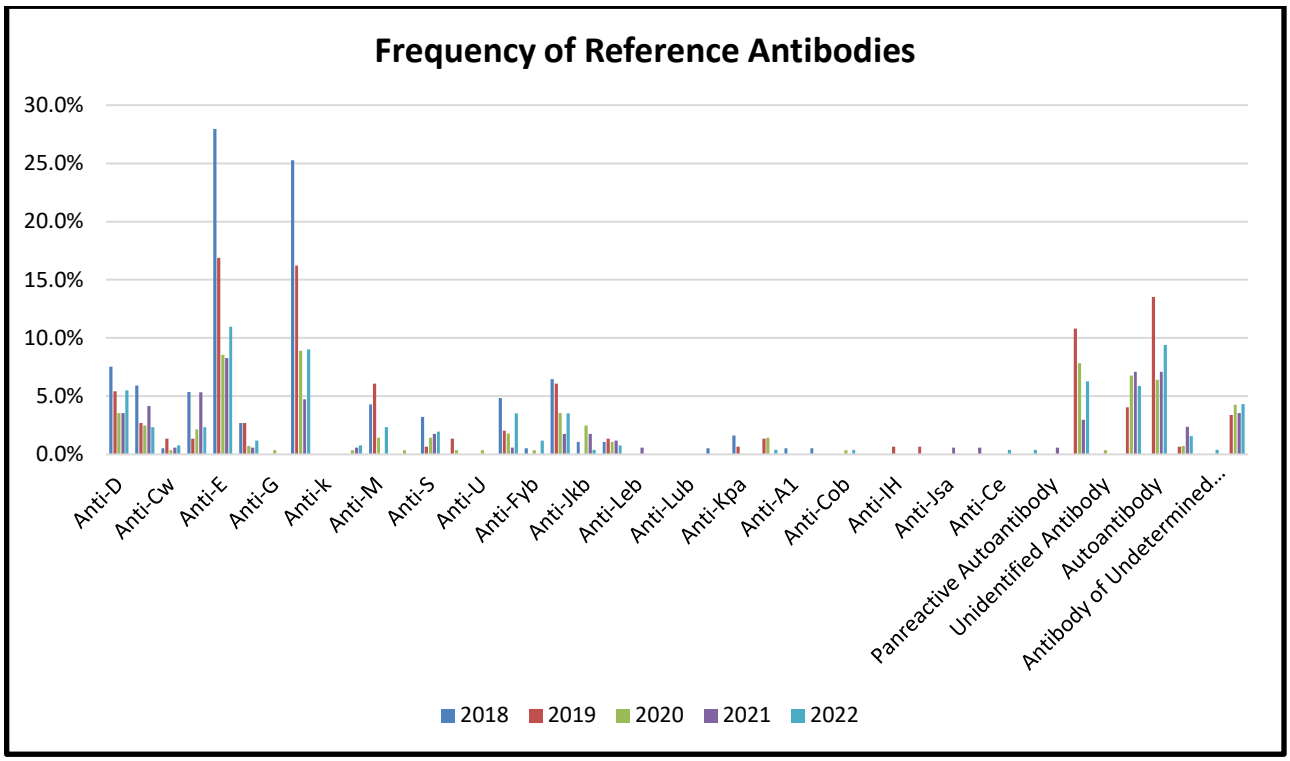


Table 7: Combination Reference Antibodies 2022

Combination Antibodies	Serology
Anti-C Anti-D	2
Anti-C Anti-D Antibody of Undetermined Significance	1
Anti-C Anti-D Cold Agglutinin Panreactive Autoantibody Unidentified Antibody	1
Anti-c Anti-E	2
Anti-c Anti-E Antibody of Undetermined Significance	1
Anti-c Anti-E Anti-Cw Anti-Fya	1
Anti-c Anti-E Anti-K	1
Anti-C Anti-E Anti-M Anti-Jka	1
Anti-Cw Anti-Fya	1
Anti-D Anti-K	1
Anti-e Anti-Ce Autoantibody	1
Anti-E Anti-Fya	1
Anti-E Anti-Fya Anti-Jkb Anti-Cob	1
Anti-E Anti-Fya Cold Agglutinin Unidentified Antibody	1
Anti-e Anti-Fyb	1
Anti-E Anti-Jka	1
Anti-E Anti-K	2
Anti-E Anti-Kpa Antibody to an HLA related antigen Unidentified Antibody	1
Anti-E Anti-Kpa Anti-Wra	1
Anti-e Anti-S Anti-Fyb Unidentified Antibody	1
Anti-E Unidentified Antibody	2
Anti-Jka Antibody of Undetermined Significance	1
Anti-Jka Cold Agglutinin Unidentified Antibody	1
Anti-K Panreactive antibody	1
Anti-K Unidentified Antibody	1
Anti-Lea Cold Agglutinin	1
Anti-M Cold Agglutinin Antibody of Undetermined Significance	1
Anti-S Anti-K	1
Anti-S Autoantibody Unidentified Antibody	1
Anti-S Cold Agglutinin Panreactive antibody	1
Anti-S Cold Agglutinin Unidentified Antibody	1
Anti-s Unidentified Antibody	1
Autoantibody Panreactive antibody	1
Autoantibody Panreactive Autoantibody Unidentified Antibody	1
Cold Agglutinin Antibody of Undetermined Significance	1
Cold Agglutinin Panreactive Autoantibody	2
Cold Agglutinin Unidentified Antibody	1

FETAL GENOTYPING

Canadian Blood Services in Alberta refers specimens for fetal genotyping on maternal plasma to the International Blood Group Reference Laboratory (IBGRL) of the National Health Services (NHS) in Bristol, United Kingdom. Amniotic fluid samples are rarely sent to the Versiti (formerly Blood Center of Wisconsin) for fetal genotyping. Testing on maternal blood samples is preferred because sample collection does not represent a risk to the fetus.

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 18 and 24 specimens in recent years.

Table 8a: Fetal Genotyping Results Summary

	2018	2019	2020	2021	2022
Total samples sent	**26**	**31**	**32**	**34**	**40**
# of patients tested	**21**	**28**	**28**	**28**	**34**
# of patients not requiring MFM follow-up. (Tested negative for the corresponding antigen)	**12**	**8**	**11**	**13**	**12**

Table 9b: Fetal Genotyping Results Summary 2022

Patient	Maternal Antibody	Predicted Fetal Phenotype	Follow-up Required
1	Anti-D	RhD Pos	Yes
2	Anti-D	RhD Pos	Yes
3	Anti-K	K Neg	No
4	Anti-D	RhD Pos	Yes
5	Anti-E	RhE Pos	Yes
6	Anti-D	RhD Pos	Yes
7	Anti-K	K Neg	No
8	Anti-D	RhD Pos	Yes
9	Anti-D	RhD Pos	Yes
10	Anti-K	K Neg	No
11	Anti-D	RhD Pos	Yes
12	Anti-E	RhE Neg	No
13	Anti-K	K Pos	Yes
14	Anti-c	Rhc Neg	No
15	Anti-D	RhD Pos	Yes
16	Anti-D	RhD Neg	No
17	Anti-E	RhE Pos	Yes
18	Anti-E	RhE Neg	No
19	Anti-D	RhD Pos	Yes
20	Anti-c	Rhc Pos	Yes
21	Anti-K	Inconclusive	Yes
22	Anti-D	RhD Pos	Yes
23	Anti-D	RhD Pos	Yes
24	Anti-E	RhE Pos	Yes
25	Anti-K	K Neg	No
26	Anti-K	K Neg	No
27	Anti-K	K Pos	Yes
28	Anti-K	K Neg	No
29	Anti-D	RhD Pos	Yes
30	Anti-K	K Neg	No
31	Anti-E	E Rh Pos	Yes
32	Anti-D	RhD Pos	Yes
33	Anti-D	RhD Pos	Yes
34	Anti-E	RhE Neg	No

RHD RED CELL GENOTYPING

Canadian Blood Services in Alberta provides RHD red cell genotyping for facilities in cases where the predicted RhD status of a patient cannot be determined due to discrepant, weak or inconclusive serological RhD testing. The following 2021 testing algorithm was used within Canadian Blood Services laboratories to determine which samples require RHD genotyping.

Figure 6: RhD Testing Algorithm

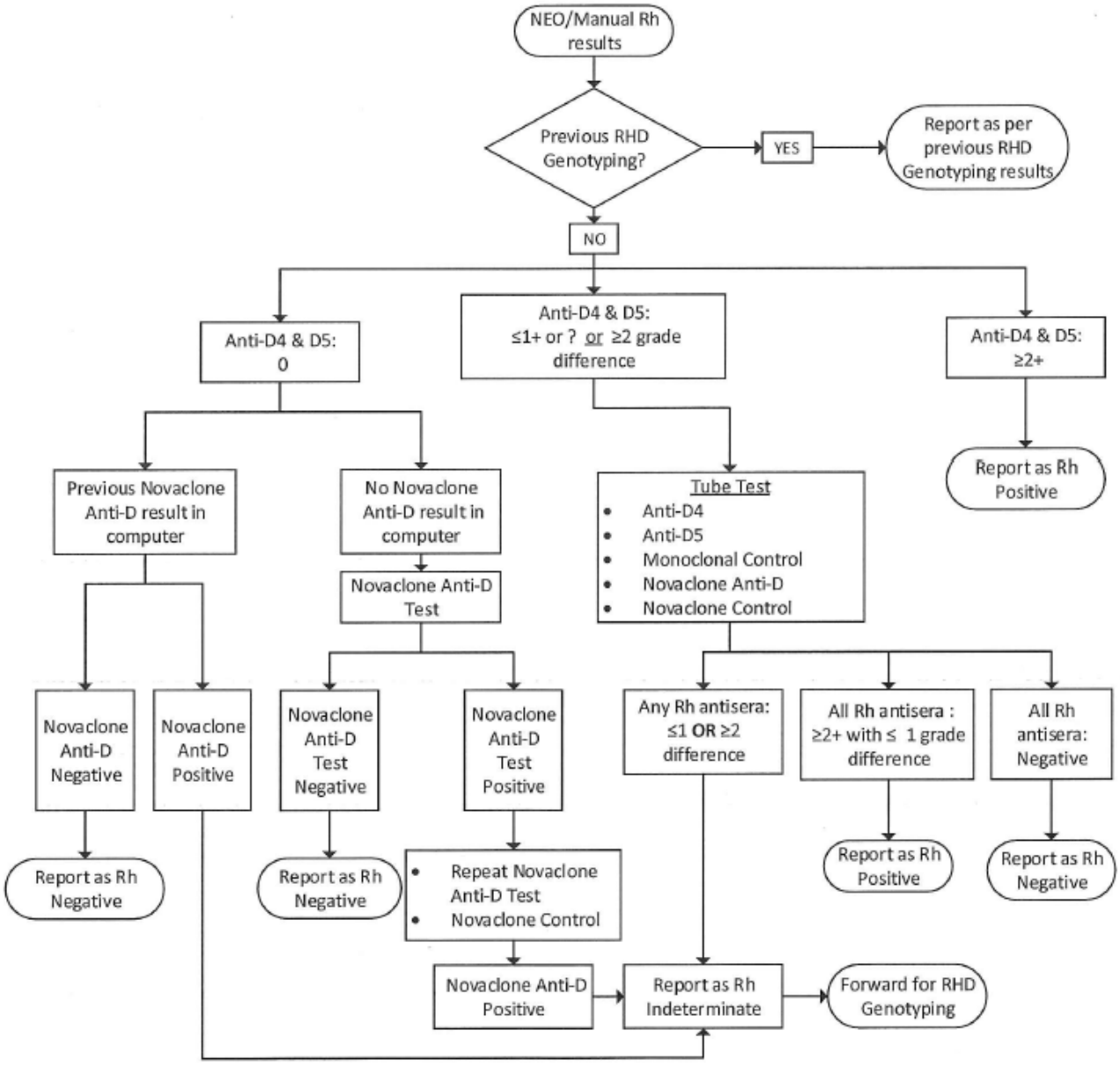


Table 10: Patient # - RHD Type/Result 2022

2022 RHD Genotyping Results	
RHD Variant	**Number Identified**
No RHD variants detected	604
Weak D type 1	223
Weak D type2	111
Weak D type 3	69
Weak D type 4.0 or 4.3	62
Weak D type 4.1	9
RHD Deletion	70
DAR	54
RHD psi (Pseudogene)	3
DAU2	10
DAU3	4
DAU4 or DV type 5	1
DAU5 or DV type1 or DBS2	2
DCSI or DFV	2
DFR, DFR2 or DFR3	3
DHMi	2
DIIIa or DIIIa-CE(4-7)-D	4
DIV	8
DOL or DOL2	1
DV type 2 or DBS1	2
DVI	5
DNB	1
DHMi	2
Del	4
Weak D type 5	4
Weak D type 11	1
Weak D type 41	1
Normal RHD with a variant allele (D+)	121
Heterozygous variant alleles (D+)	1
Heterozygous variant alleles (D-)	34
Sent for Sequencing	111
Total	**1529**

Table 10a: *RHD* Genotyping – Number of Samples Tested by Year

Year	2018	2019	2020	2021	2022
Total # samples tested	734	982	1146	1459	1529

Table 10b: *RHD* Genotype Testing by Province in 2022

Province	No. of Samples Tested	% of Samples
Alberta	241	16
British Columbia	124	8
Manitoba	25	<2
New Brunswick	11	<1
Newfoundland and Labrador	11	<1
Nova Scotia	24	<2
Ontario	995	65
Prince Edward Island	5	<1
Saskatchewan	93	6
Total Tested	**1529**	**100**

The Diagnostic Services Lab in Edmonton receives samples for *RHD* genotyping from across Canada (excluding Quebec). The assay, Immucor's BioArray Molecular BeadChip™ Test, is a targeted assay designed to detect the most common variants of the *RHD* gene. Interrogating 35 genetic markers, the assay can make 68 variant allele calls. The most common variant *RHD* alleles detected are *weak D type 1, weak D type 2*, and *weak D type 3*. Individuals carrying these alleles can be safely treated as RhD positive as it has been established that they will not form alloanti-D. Current national and international guidelines recommend that patients with any other weak or partial D genotype should be treated as RhD negative although it must be recognized that the alloimmunization potential of many of these weak D variants is uncertain.

When none of the interrogated allele variants are detected, the assay returns a result of "Possible D". Based on the findings of a quality assurance study of "Possible D" samples sent for *RH* Next Generation Sequencing (NGS), a change was made to the interpretation and reporting of results for the *RHD* genotyping assay. As of October 2022, all prenatal patients with weak or discrepant RhD serologic phenotype whose genotyping results show no detectable *RHD* variants will be interpreted as RhD negative with the recommendation that they be considered Rh immune globulin (RhIG) eligible.

The testing and reporting strategy for hemoglobinopathy patients is different. Current guidelines recommend that all hemoglobinopathy patients who serologically test as RhD positive should have *RHD* genotyping performed in conjunction with extended red cell antigen genotyping that includes an assessment of *RHCE* variants. (At Canadian Blood Services this is performed using Progenika's ID Core XT assay). In the absence of weak or discrepant D serologic phenotype or unexplained genetic markers in the assay results, these patients will be interpreted as RhD positive when no *RHD* variants are detected by the Immucor BioArray Molecular BeadChip™ Test. Samples with discordant serologic findings or unexplained assay results may be referred out for *RH* gene sequencing.

The field of blood group genomics has expanded significantly over the past five years with the identification of an ever-increasing number of new *RHD* alleles and increased recognition of the remarkable *RH* genetic diversity across different ethnic groups. To better reflect the limitations of this targeted assay in the ethnically diverse Canadian population, samples returning a result of "possible D" will be reported as "No *RHD* variants detected". In all cases, the results of *RHD* genotyping must be correlated with the serologic findings and clinical context of the patient. Medical consultation is available at CBS reference labs to assist with result interpretation and the need for additional testing will be assessed on a case-by-case basis.

MONOCYTE MONOLAYER ASSAY (MMA)

The Monocyte Monolayer Assay (MMA) is a laboratory test that provides an in vitro assessment of the clinical significance of red cell antibodies. In cases where only crossmatch incompatible red cell units are available for transfusion, the MMA helps to select the most appropriate components.

The first step of the MMA is to sensitize selected donor red cells with the patient's plasma containing the identified red cell antibody(ies). Next, the sensitized red cells are mixed with a source of allogeneic monocytes. Red cells which have been phagocytosed (ingested) by the monocytes are then counted using a microscope. This count will help to determine the likelihood of survival if the red cells were transfused.

The MMA has been available for patients mainly in the Toronto area from Dr. Donald Branch's research laboratory. Canadian Blood Services has since optimized Dr. Branch's method for use in a clinical laboratory setting. New equipment was purchased, operating procedures were written, training/competency programs were created, and validation testing was performed. The test was implemented in the Edmonton Diagnostic Services lab in July 2022.

Currently the MMA test is not an orderable test, but rather is only available through consultation with a CBS Transfusion Medicine Physician and the Rare Blood Program. The MMA results are not reported directly to the requesting physician or facility, but are correlated and reported together with the patient's red cell genotyping results and results from the serological investigation performed at the National Immunohematology Reference Lab.

Since implementation, two patient samples have been tested in the Edmonton lab to aid in the selection of red cell units for transfusion.

Table 111: Number of MMA Tests

Samples Tested	Antibody	Number of Red Cell Components Assessed for Transfusion
Patient #1	Anti-Kpb	8
Patient #2	Suspected HLA	10

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 reference specimens has consistently met the predefined TAT threshold. Samples whose testing failed to meet expected TATs are usually those where clinically significant antibodies are detected or where difficulty in finding compatible blood is encountered.

Table 12: Turnaround Time – Routine Criteria by Specimen Type

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

Table 13: Turnaround Time – Perinatal Routine TAT

Turnaround Time (TAT)	2018	2019	2020	2021	2022
% of Specimens Tested within 72 hours	88%	85%	85%	83%	82%
% of Specimens Tested > 72 hours	12%	15%	15%	17%	18%

Figure 7: Perinatal Routine TAT

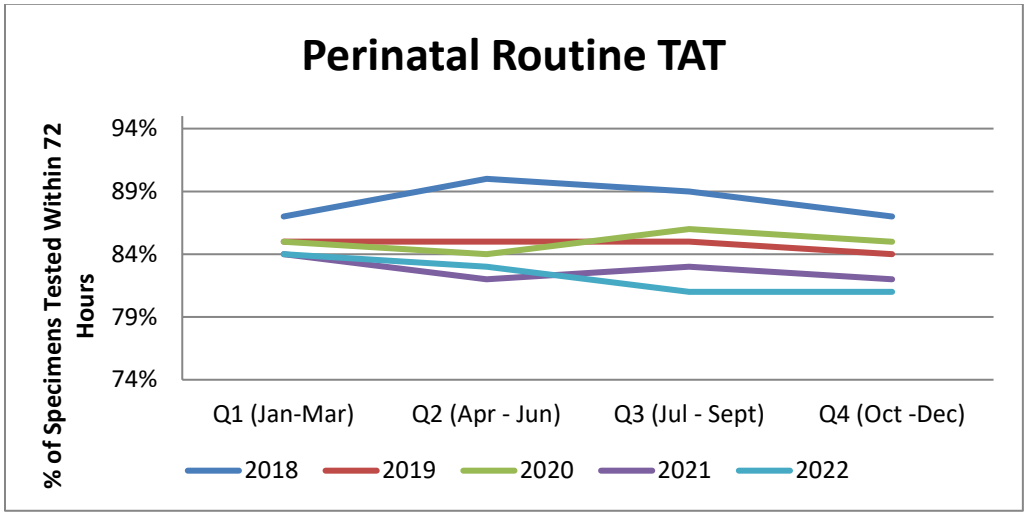
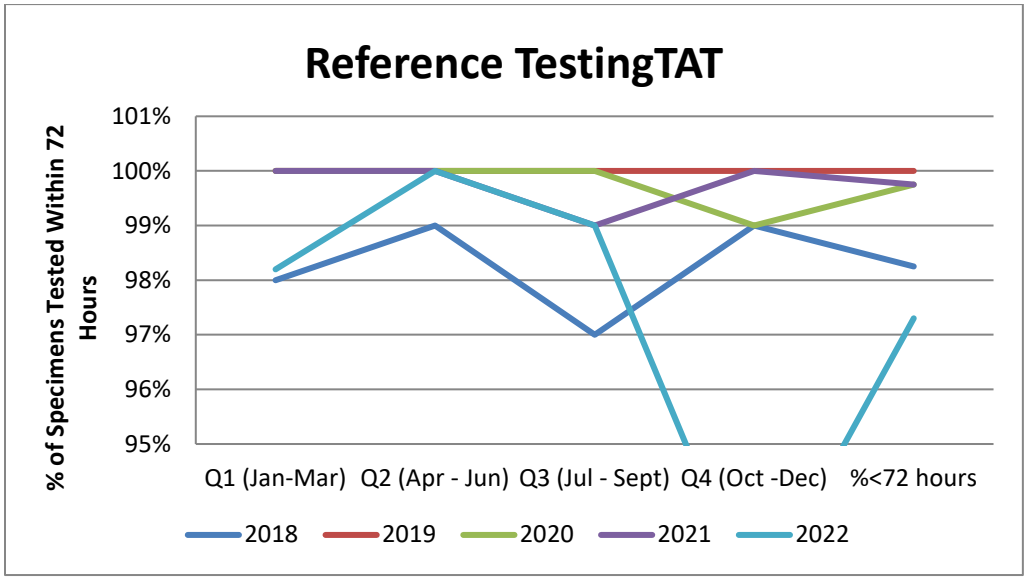


Table 124: Turnaround Time – Reference Specimens

Turnaround Time (TAT)	2018	2019	2020	2021	2022
% of Specimens Tested within 72 hours	98%	100%	100%	100%	97%
% of Specimens Tested > 72 hours	2%	0%	0%	0%	3%

Figure 8: Reference TAT



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 for both 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 reference and the perinatal laboratories are somewhat different.

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 15: Quarterly Rejection Rates – Perinatal Specimens 2022

Rejection Category	Q1 (Jan-Mar)	Q2 (Apr - Jun)	Q3 (Jul - Sept)	Q4 (Oct - Dec)
Requisition	27	41	31	44
Specimen	120	84	119	201
Discrepancies Between Requisition & Specimen	20	11	9	9
Discrepancies Between Current Requisition & Historical Records	0	0	0	0
Other (Duplicates, etc.)	4	8	1	208*
Total # specimens rejected	171	144	160	462
Total # specimens received	16442	15722	15004	15974
Rejections as a % of total	1.0%	0.9%	1.1%	2.9%

* There were a high number of rejected samples in Q4 due to improper storage. An investigation was performed to mitigate recurrence of this issue. Sample recollection was requested on all impacted patients.

Figure 9: Perinatal Rejection Reasons 2022

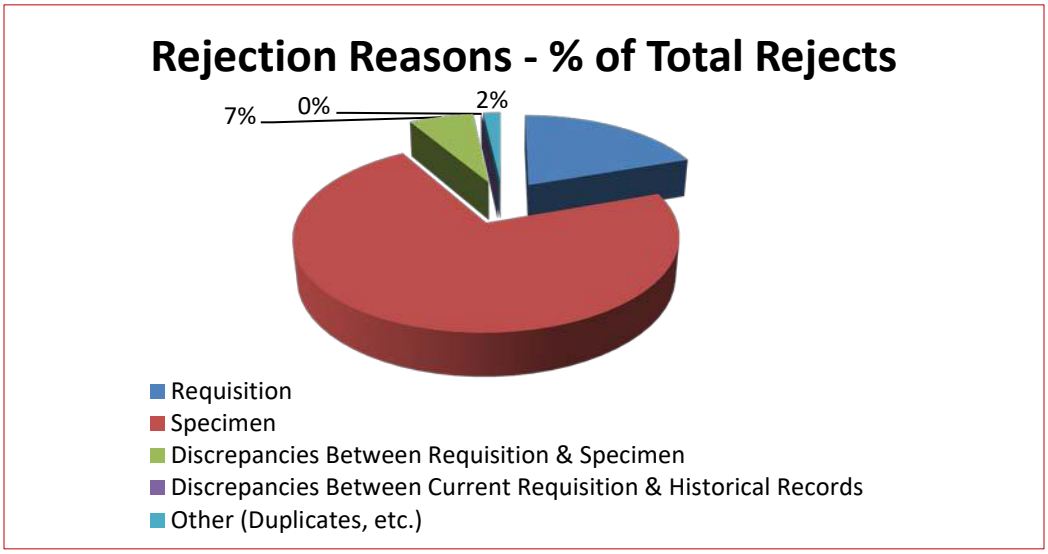


Table 16: Quarterly Rejection Rates – Reference 2022

Rejection Category	Q1 (Jan-Mar)	Q2 (Apr - Jun)	Q3 (Jul - Sept)	Q4 (Oct -Dec)
Requisition	2	1	0	1
Specimen	17	13	13	17
Discrepancies Between Requisition & Specimen	0	0	2	0
Discrepancies Between Current Requisition & Historical Records	0	0	0	0
Other (Duplicates, etc.)	10	7	1	3
Total # specimens rejected	29	21	16	21
Total # specimens received	**465**	**435**	**480**	**461**
Rejections as a % of total	**6.2%**	**4.8%**	**3.3%**	**4.6%**

Figure 10: Reference Rejection Reasons 2022