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ACCREDITED
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SURVEY DESCRIPTION/OBJECTIVE

PRENATAL PATIENT WITH INCREASING LEVEL OF ANTIBODY, PRE-OPERATIVE PATIENTS; TITRATION; POSITIVE DAT; CROSSMATCH

The survey consisted of samples representing four patients and a donor unit for crossmatching. Participants were instructed to perform ABO/Rh, antibody detection, direct antiglobulin test (DAT), antibody identification, antibody titration, phenotyping where appropriate and crossmatching.

NEW FOR THIS SURVEY

Participants were able to report the following: ABO and Rh grouping results for the donor unit, repeat ABO and Rh grouping results for the patient samples, antibody detection results for individual screening cells, whether a computer crossmatch was performed, and phenotyping for K antigen for the donor unit.

SURVEY PARTICIPATION

There were 89 submissions out of 89 participating laboratories for TMED A material (73 Ontario and 17 voluntary). All participants submitted results on time.

DESCRIPTION OF TESTING MATERIAL

Vial	Clinical History	Group	DAT	Antibody
TMED-1509-A-1	This sample is from a 35-year-old prenatal patient taken at 20 weeks gestation. This is her second pregnancy. She has never received any blood products.	A Rh Negative	Negative	Anti-D (1:4-8)
TMED-1509-A-2	This sample is from the same prenatal patient described for TMED-1509-A-1, now at 28 weeks gestation.	A Rh Negative	Negative	Anti-D (1:32-64)
TMED-1509-A-3	This sample is from a 14-year-old male patient scheduled for a splenectomy. The patient has never received any blood products.	B Rh Positive	Negative	None
TMED-1509-A-4	This sample is from a 45-year-old male patient who has been recently transfused (within the last 3 months). The historical record indicates that anti-K was previously identified.	O Rh Positive	Positive (IgG)	None
TMED-1509-A-5	This is a donor unit available for crossmatch to patients TMED-1509-A-3 and TMED 1509-A-4.	O Rh Negative, K-	Negative	NA

STATUS OF TESTING MATERIAL QUALITY

Homogeneity and stability testing indicated the material met the pre-determined criteria for acceptability as a Proficiency Testing challenge.

Courier service records confirmed that the samples were delivered within two days after distribution to 99% of participants. Ninety-eight per cent of participants indicated they received the material within two days of distribution.

Ninety-nine per cent of participants indicated the material arrived in satisfactory condition. One site reported leakage of one vial.

VALIDATION OF ASSIGNED VALUE AND ALLOWABLE RANGE OF RESULTS

The assigned value for all tests is determined by the mode of participant responses. Where there is no mode, the assigned value is determined by the median. In consideration of the variety of testing platforms and methods in use, an allowable range of results is determined. For DAT the assigned values were established according to the mode by method.

PERFORMANCE ASSESSMENT

Participants were assessed on all test responses. Responses were reviewed prior to survey analysis for consensus and for any indication of material deterioration. All comments on worksheets were reviewed for consideration.

TMED-1509-A-1

This sample was from a 35-year-old prenatal patient taken at 20 weeks gestation. This was her second pregnancy. She had never received any blood products. The sample was A Rh negative with anti-D present, at a titre of 1:4–1:8, and the DAT was negative.

- There were no discordances for ABO or Rh grouping and interpretation; 33 sites repeated the ABO and Rh groupings.
- There were no discordances for antibody detection.
- There were no discordances for antibody identification; all participants identified anti-D.
  - A number of sites reported that other clinically significant antibodies could not be excluded.

Antibody	-E	-C	-Cw	-K
Count	8	6	1	1

- Fifty-five sites performed the requested antibody titration; the titre was previously confirmed to be 4–8 by tube following the AABB method.
  - Results reported by column agglutination (Gel) ranged from 8 to 128 (no mode or median)
  - Results reported by tube method ranged from 4 to 64 (mode 16).

Titres	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**Total**
Gel	0	0	0	2	1	1	1	1	6
Tube	0	0	1	9	**26**	11	2	0	49

Participants were also asked to report on their methods:

Diluent		**Indicator cells**		**Incubation Time**		**AHG**		**End Point**	
Tube Method									
Saline	45	R1r	1	30 min	26	Poly AHG	9	1+ macroscopic	48
5% Alb	2	R1R1	5	45 min	6	Anti-IgG	39	1+ microscopic	1
NR	2	R2R2	39	60 min	17	NR	1		
		R0r	3						
		RzR1	1						
Gel Method									
		R2R2	5	15 min	5			1+ macroscopic	5
		R0r	1	NR	1			Last column demonstrating a weak reaction	1

- There were no discordances for DAT.
- No phenotyping was required for this sample; however, a number of sites reported the results of antigen typing.

Antigen	E	C
Count	6	5

TMED-1509-A-2

This sample was from the same prenatal patient described for TMED-1509-A-1, now at 28 weeks gestation. The sample was A Rh Negative with anti-D present, at a titre of 1:32–1:640, and the DAT was negative.

- There were no discordances for ABO or Rh grouping and interpretation; 16 sites repeated the ABO and Rh groupings.
- There were no discordances for antibody detection.
- There were no discordances for antibody identification; all participants identified anti-D.
  - A number of sites reported that other clinically-significant antibodies could not be excluded.

Antibody	-E	-C	-Cw	-K
Count	8	6	1	1

- Fifty-five sites performed the requested antibody titration; the titre was previously confirmed to be 32–64 by tube following the AABB method. Participants were expected to report a ≥2-tube dilution difference in titres between samples TMED-1509-A-1 and -A-2.
  - Results reported by column agglutination (Gel) ranged from 32 to 512 (no mode or median).
  - Results reported by tube method ranged from 32 to 512 (mode 128).

Titres	32	64	128	256	512	Total
Gel	1	1	1	2	1	6
Tube	6	10	**24**	7	1	48

  - 51/55 laboratories (93%) reported a ≥2- tube dilution difference in titres between samples TMED-1509-A-1 and -A-2.
  - Four laboratories reported a 1-tube dilution difference between the samples.
- There were no discordances for DAT.
- No phenotyping was required for this sample.

TMED-1509-A-3

This sample was from a 14-year-old male patient scheduled for a splenectomy. The patient had never received any blood products. The sample was B Rh Positive. There were no unexpected antibodies and the DAT was negative.

- There were no discordances for ABO or Rh grouping and interpretation; 38 sites repeated the ABO and Rh groupings.
- There was no antibody present; however, one site reported the antibody screen as Positive and that they were unable to identify the antibody.
- There were no discordances for DAT.
- No phenotyping was required for this sample.
- The patient was eligible for both Immediate Spin (IS) and computer (electronic) crossmatching.
  - Eighty-five sites performed the crossmatch for this sample; 47 by IS, 28 by computer and 10 by AHG methods.
  - There were no discordances for crossmatching.

TMED-1509-A-4

This sample was from a 45-year-old male patient who had been recently transfused (within the last three months). The historical record indicated that anti-K was previously identified. The sample was O Rh positive. There were no unexpected antibodies; however, the DAT was positive with IgG.

- There were discrepancies for both ABO and Rh grouping.
  - On initial and repeat testing one site incorrectly reported 0 with anti-D and reported the Rh interpretation as negative.
  - On repeat testing one site interpreted the ABO group as B.
- There were no discordances for antibody detection and antibody identification was not required.
- All sites detected the positive DAT; there were no discordances.
- Phenotyping was not required and also not appropriate since the patient had been transfused within the last three months
  - Two sites type this sample for K antigen.
- The patient was not eligible for either Immediate Spin (IS) or computer (electronic) crossmatching.
  - Three sites appear to have performed AHG crossmatches but omitted reporting actual test results.

TMED-1509-A-5

This sample was the donor unit available for crossmatch to patients TMED-1509-A-3 and TMED-1509-A-4. It was O Rh Negative, K-.

- There were no discordances for ABO or Rh grouping and interpretation.
- There were no discordances for phenotyping.

SUPPLEMENTAL QUESTIONS

You perform Rh (D) typing on a sample from a prenatal patient and obtain 1+ with anti-D (by IS or Gel).

1. How would you interpret the Rh (D) type?

a)	Rh (D) positive	14	16%
b)	Rh (D) negative	7	8%
c)	Unable to interpret	68	76%

2. If unable to interpret, what further testing would you perform?

a)	Refer out for confirmation of D typing	9	10%
b)	Test for weak D	48	54%
c)	Other (specify):	17	19%
	Send out for Rh Genotyping	2	
	Perform tube testing using 15 min room temperature incubation as per manufacturer instructions. If result continues to be <2+ in strength, perform Weak D testing.	2	
	Test for weak D and depending on the result obtained; refer out for confirmation of D typing.	2	
	Add a comment that patient is a "weak D" and the implications	1	
	Would test in tube against two anti-D's - if discrepancy exists in reactions between the two anti-D's then patient would be called Rh negative until genetic testing results can be obtained.	1	
	Test other sources of commercial anti-D.	3	
	Rh enhancement testing	9	
	No reply	15	17%

3. If this patient required a transfusion of red cells, what Rh group would you select?

a)	Rh (D) positive	22	25%
b)	Rh (D) negative	67	75%

4. According to your lab's policies/procedures, would this patient be a candidate for RhIg?

Yes	56	63%
No	30	34%
No reply	3	3%

5. Would you send this sample to a reference lab for genotyping?

Yes	40	45%
No	47	53%
No reply	2	2%

The following questions pertain to transfusion practice for women of childbearing age:

6. Does your laboratory have a policy to type all female patients of childbearing potential for K antigen?

Yes	3	3%
No	86	97%

7. Does your laboratory have a policy to provide K(-) red cells for transfusion to patients of childbearing potential?

Yes	2	2%
No	87	98%

Site	Supplementary Comments
1	Question # 5 - We do not automatically send a sample out for RhD genotyping but would recommend this test to the Physician and place a comment on the report. Specimen will be referred to Canadian Blood Services
2	1. We would notify the physician treating her as Rh negative until the report for Rh genotyping came back.
3	If we obtained a 1+ reaction with Anti-D, we would not interpret the result until we did further testing.
4	Question #3 - while RH type was undetermined, patient would be transfused RH negative blood, until the type was resolved.
5	We are currently in the process of revising our policy/procedures for the interpretation and subsequent follow up tests including genotyping for women of childbearing age who have weaker than expected results with anti-D reagents.
6	#3 and #4 Depending on weak D testing how the RH would be reported and if the patient is a candidate for RHIG.
7	Recently discussed with the laboratory medical director to implement giving Kell negative blood to all females of child bearing age. Policy is in-process.
8	Question 3: Rh selection dependant on results of Weak D testing. Question 4: Rhig candidacy dependant on results of Weak D testing.
9	Question#4: RhIg would be administered upon doctor's discretion.
10	If the patient is Rh Positive (Weak) we do not call them a candidate for Rh Immune globulin. The decision is left up to the Most Responsible Physician. At this time we are not sending them out for genotyping.
11	For question#3: Rh negative blood would be provided if the transfusion is required before the resolution of Rh typing. For question#4: RhIg candidacy dependent on the results of the weak D typing and genotyping.
12	Questions 3 to 5. The patient would be treated as Rh negative until genotyping is known.
13	Regarding questions 3, 4 and 5: The decision made would initially be based on the result of the weak D testing. If suspect for a partial D, the patient would be considered Rh Negative and a sample would be sent for genotyping to determine the patient's Rh status. The results of the genotype would determine if Rh immune globulin is warranted.
14	Question #4: Issue of RhIg would be re-assessed after the result for genotyping is received. If genotype would come back as; patient will not develop ant-D, then will not issue RhIg in the future.
15	Regarding question 4: Based on CBS (genotyping) findings some patients may be upgraded to Rh Positive status and therefore not require RHIG. Others would be classified as Rh Negative and therefore receive RHIG. We would follow Pathologist recommendations.
16	Rh enhancement is done by incubating D testing at room temperature for 5 minutes. Strength of this determination is whether patient is considered Rh Pos (>or= to +2) or Neg.
17	Question 5: Sample would be sent to CBS for confirmation testing. CBS would determine if further genotyping is warranted.
18	Question 5. We would only refer a sample for genotyping on the advice of a hematopathologist. Our reference lab requires unopened samples so the patient would need to be recollected.

DISCUSSION

ANTIBODY IDENTIFICATION

All participants correctly identified anti-D in samples TMED-1509-A-1 and TMED-1509-A-2; the reactivity reported was 2–3+ and 3–4+, respectively.

Four laboratories questioned the possibility of anti-D due to RhIg, despite the history given that the patient had not received any blood products. Anti-D due to RhIg would be unlikely with such strong reactivity in the antibody screen and antibody titre results. Passively acquired anti-D rarely achieves a titre above 4. In some cases passive anti-D due to RhIg can be distinguished from anti-D formed by recent alloimmunization, by treating the sample with DTT to inactivate the IgM component present in active anti-D. Anti-D due to RhIg cannot be inactivated by DTT treatment as it is entirely IgG.[1] A number of participants were unable to exclude anti-E, anti-C, anti-Cw and anti-K in both samples.

Sample TMED-1509-A-3 had no antibodies; however, one laboratory reported 1+ reactivity with SCIII.

Sample TMED-1509-A-4 was from a recently transfused patient with a history of anti-K, non-reactive at present. All laboratories correctly reported the antibody screen as negative.

ANTIBODY TITRATION

The reproducibility of antibody titres is a known problem demonstrated in previous surveys. Variability of titre results can be an issue especially for the determination of changes in titres (increase) during the pregnancy. The majority of laboratories used the tube test for the two antibody titrations performed for this survey. The most commonly used methods included the use of anti-IgG, an R_2R_2 indicator cell and saline as the diluent. The end point was a 1+ macroscopic reading. More than 50% reported a median titre of 16 (8–32 range) in sample TMED-1509-A-1 and a median titre of 128 with a range of 32–512 for sample TMED-1509-A-2. Six laboratories using the Gel method reported a much larger range of reactivity.

The purpose of this survey was to assess proficiency in antibody titration by testing samples TMED-1509-A-1 and TMED-1509-A-2, with increasing titres. Ninety-three per cent of laboratories reported a two-tube or greater increase in titres, which was the expected result.

Selection of the most suitable red cells to use for titration is controversial. Some suggestions are to use the strongest expression of antigen such as R2R2 for anti-D titration while others choose red cells with an expression of single dose antigen, such as R1r, to mimic the phenotype expressed in fetal circulation. The selection of R2R2 cells is most practical since screening cells instead of panel cells can be used. The main focus should be on reproducibility achieved by consistently using the same red cell phenotype.[3]

It is important to note that even if the antibody reactivity seems to remains the same in the antibody screen, the titration should be repeated to monitor the changes in anti-D titres.

DAT

The DAT on sample TMED-1509-A-4 was reported positive 2–3+ by tube testing. Most laboratories reported positive with AHG/ IgG and all laboratories that tested with anti-C3 reported it negative.

Gel DAT was reported stronger, reacting 3–4+. The DAT requirement is to test for both IgG and C3 on red cells preferably by performing the initial DAT using polyspecific AHG, and only if positive to test with monospecific anti-IgG and C3. Going directly to IgG and C3 is less cost effective.[2]

PHENOTYPE

Two laboratories performed K typing on sample TMED-1509-A-4, a recently transfused patient. Phenotype should be performed only on patients with no transfusion during the past three months. Antigen typing on transfused patients may result in erroneous, unreliable results since a mixed population of patient and donor cells is being tested.

CROSSMATCHING

Crossmatch was performed on two samples. Sample TMED-1509-A-3 had no antibody present and did not require a serological AHG crossmatch. From 85 participants reporting, 28 performed electronic crossmatch; all performed a repeat ABO/D, most on a same patient sample, one participant would draw a new sample and test it prior to crossmatching. Sample TMED-1509-A-4 was from a patient with previously identified anti-K, non-reactive at present, and was not eligible for immediate spin or electronic crossmatch. This patient requires a full serological AHG crossmatch using K negative donors. Some laboratories performed and reported both IS and AHG crossmatch as per their policy.

SUPPLEMENTARY QUESTIONS

Serological weak D is defined as reactivity <2+ with commercial anti-D reagent. The significance of weak D testing on donors is straightforward; donors with weakened D antigen expression are considered Rh(D) positive. It is also a standard practice to perform weak D testing on newborns and consider them Rh(D) positive in relation to RhIg prophylaxis.[4] The weak D testing on patients is much more complicated and controversial, and is usually performed to resolve discrepancy between results with different anti-D antisera or methodology, i.e. manual tube test versus automation.

The most common weakened D antigens seen in laboratories are weak D and partial D. The location of the amino acid change, either intracellular or extracellular, has an impact on the number of antigen sites. Weak D has a reduced number of D antigen sites on red cells that can be further weakened by C antigen in trans position to a weak D. Weak D changes are most likely located intracellularly or in the transmembrane region of the D antigen and are unlikely to form anti-D. Partial D phenotype (also known as variant D phenotype) is associated with amino acid substitution of RhD protein and lack of D epitope. These changes are located extracellularly on the outer surface of red cells and have a potential to produce anti-D. [5,6,7]

The possible anti-D alloimmunization in partial D patients should be taken into consideration as it can cause a significant problem for female patients of child bearing potential (45 years old and younger). To prevent sensitization to D antigen for females in this group Rh(D) negative red cells should be crossmatched and RhIg given as per perinatal protocol for Rh negative pregnant females.[5,6,7]

Several (76%) laboratories would not interpret the results with reactivity of only 1+ with anti-D reagent, 54% of these would perform a weak D test as a follow up. Seventy-five per cent of laboratories stated they would transfuse Rh(D) negative red cells and 63% would consider these patients to be candidates for RhIg.

Rh(D) Genotype is a useful tool to differentiate between weak and partial D but is not routinely available in most laboratories. The test can be referred out to the CBS.

Consideration to prevent anti-K sensitization has been a focus of discussions at several conferences recently. Anti-K is a difficult antibody to monitor during the pregnancy and prevention would be beneficial. The pathogenesis of Hemolytic Disease of Fetus and Newborn (HDFN) due to anti-K is very different from anti-D. Anti-K HDFN is associated with a lower degree of hemolysis and the fetal anemia results predominantly from suppression of erythrocytes.[8]

The question of typing the female patients of childbearing potential and crossmatching K antigen negative red cells is debatable. Should we consider transfusing K negative red cells regardless of K type status of patients due to a high incidence of negativity for the K antigen? Survey results showed that only three laboratories perform K typing and two laboratories would crossmatch K negative red cells.

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