NAC STATEMENT ON RBC GENOTYPING

RBC GENOTYPING SUBCOMMITTEE

RBC Genotyping Subcommittee Members:	Tanya Petraszko, MD; Chair (NAC, CBS) Jennifer Fesser, MD; (NAC) Arjuna Ponnampalam, MD; (NAC) Meer-Taher Shabani-Rad, MD; (NAC) Gwen Clarke, MD; (University of Alberta & Island Health) Philip Berardi, MD, PhD; (Ottawa Hospital) Celina Montemayor-Garcia, MD, PhD; (CBS)
NAC Chair:	Andrew Shih, MD
Provincial Ministry Representative:	Katherine Logan (BC)
NAC Coordinator:	Kendra Stuart
Publication Date:	May 9, 2024

Cite As:

Petraszko T, Fesser J, Ponnampalam A, Shabani-Rad MT, Clarke G, Berardi P, Montemayor-Garcia C. NAC Statement on RBC Genotyping [Internet]. Ottawa: National Advisory Committee on Blood and Blood Products; May 9, 2024 [cited YYYY MM DD]. Available from: https://nacblood.ca/en/resource/nac-statement-rbc-genotyping

TABLE OF CONTENTS

ABBREVIATIONS

CBS	Canadian Blood Services
DAT	Direct Antiglobulin Test
DTT	Dithiothreitol
NGS	Next Generation Sequencing
RBC	Red Blood Cell
SCD	Sickle Cell Disease
PCR	Polymerase Chain Reaction

DEFINITIONS

Alloimmunization – an immune response to foreign antigens after exposure to genetically different cells or tissue – in this context referring to foreign antigens on red cells to which a person is exposed through transfusion or pregnancy.

Alloantibody – an antibody directed against red cell antigen different from those in the recipient/patient.

Antisera – reagents containing antibodies used in serological testing to determine the presence or absence of specific red cell antigens (or the red cell antigen phenotype).

Autoadsoprtion – advanced serological methodology to differentiate between multiple antibodies (allo and autoantibodies) in patient/recipient plasma using patient/recipient's own cells.

Autoantibody – an antibody directed against a red cell antigen present on the recipient/patient red blood cells.

Chronic transfusion therapy – the requirement for ongoing red cell transfusion support at regular intervals over a prolonged period.

Genotyping assays – laboratory tests that detect differences in DNA that can lead to changes in phenotype. These tests may be used in pretransfusion testing to predict red cell antigen types.

Hemoglobinopathy – a group of inherited disorders in which there is abnormal production or structure of the hemoglobin molecule resulting in chronic anemia.

High prevalence antigens – red cell antigens present on the red cells of >99% of individuals.

Low prevalence antigens – red cell antigens present on the red cells of <1% of individuals.

Next generation sequencing – DNA sequencing method that sequences multiple fragments in parallel, allowing for higher volume sequencing.

Non-ABO antigens – RBC antigens from one or more blood group systems other than the ABO system (e.g. Rh, Kell, Kidd or Duffy blood group antigens are non – ABO antigens).

Pan-reactive – antibodies in patient/recipient plasma that react broadly to all reagent red cells tested.

Private antibodies – antibodies arising in a prenatal individual and directed against low prevalence, paternally inherited antigens on a fetus or neonate. These antibodies are not detected on a standard antibody screen as the antigen targets are rarely present (low prevalence). The particular antigen antibody combination is private to the unique maternal paternal and fetal unit, is generally suspected based on a positive neonatal direct antiglobuin test with anemia.

RBC genotype – The DNA code associated with blood group antigens.

RBC phenotype – The antigens expressed on the RBC surface. These may be detected using serological tests or predicted using genotyping assays.

Sanger sequencing – DNA sequencing method that only sequences a single DNA fragment at a time. Gold standard methodology for DNA sequencing.

Serological discrepancies – situations where different antisera result in different interpretations of antigen presence or absence.

Serological test – a method of laboratory testing based on antigen antibody reactions and their detection. These tests may be used to identify red cell antigens (phenotype) or antibodies in pre transfusion testing.

INTRODUCTION

Alloimmunization to red blood cell (RBC) antigens is a clinically meaningful outcome of transfusion or pregnancy that is not uncommon. Alloantibodies are seen in up to 3% of generalized hospital patients in one United States study,[1] and in up to 80% of chronically transfused hemoglobinopathy patients, most notably Sickle Cell Disease (SCD).[2]

Reduction of alloimmunization can be achieved by prophylactic matching of non-ABO antigens between donor and recipient for chronically transfused populations. Additionally, in patients who are already alloimmunized, therapeutic matching of non-ABO antigens is necessary to prevent the hemolytic consequences of transfusion and to facilitate appropriate transfusion care. Serologic methods of blood group determination for patients are inherently limited by insensitivity to minor structural antigen variations that are clinically meaningful, lack of commercially available reagents for rare antigens, and challenges in distinguishing between donor and recipient cells in recently transfused individuals.[3]

Genotyping assays overcome these limitations and allow for effective blood group antigen determination. Current advances in the field demonstrate a high concordance rate between genotyping assays and serologic methods, allowing for more widespread adoption of this methodology.[4] However, limitations including cost and availability make it difficult to ensure equitable access for all patients who may benefit, and there is no single published guideline to identify the patients who would most benefit from this testing.

This document serves to describe clinical scenarios in which genotyping could be considered to predict phenotypes facilitating optimal RBC matching to avoid alloimmunization, or to select the safest RBC units for transfusion in patients who are already alloimmunized. Genotyping results should always be interpreted in conjunction with the patient's clinical context, serologic phenotype, and alloimmunization history. Discordances should be reported to the National Immunohematology Reference Laboratory for further investigation and resolution.

Current State in Canada

Red cell genotyping for non-ABO and RH antigens is offered at Canadian Blood Services (CBS) and The Ottawa Hospital. The RBC Genotyping Subcommittee is unaware of any other provider of genotyping for red cell antigen genes in Canada outside of the province of Québec.

DNA arrays for extended RBC typing do not routinely include ABO antigens and genotyping to predict ABO antigens for transfusion purposes is not currently offered in Canada. When required, this testing is available at international reference laboratories using validated laboratory developed tests. This approach requires assessment of multiple alleles and currently employs Sanger sequencing or next generation sequencing (NGS) targeted to the ABO genomic locus.

ABO testing is increasingly being performed outside of transfusion medicine laboratories, by university or clinical HLA laboratories as part of organ transplantation programs. This testing has not yet been validated in the transfusion medicine setting. Ambiguity for ABO genotyping must be interpreted with care and ABO genotyping for transfusion purposes requires expert

interpretation. Importantly, serologic testing for ABO remains the standard of care and ABO discrepancies in pre-transfusion patients should be managed by provision of group O blood.

Canadian Blood Services

CBS offers extended red cell antigen genotyping through the Health Canada licensed IDCOREXT assay. This assay is based on probe hybridization of targeted PCR products coupled to flow cytometry, and it predicts the expression of 37 blood group antigens in 10 blood group systems. In addition, CBS offers *RHD* and *RHCE* genotyping through the Health Canada licensed Immucor BeadChip Kit. The turn around time for these three genotyping tests is up to 14 days. For complex samples that cannot be resolved with these targeted genotyping platforms, CBS offers blood group gene sequencing (Sanger or NGS) through an international referral. These services are available to all Canadians outside of Québec.

The Ottawa Hospital

Genotyping for non-ABO and *RHD* is offered at The Ottawa Hospital using the HEA BeadChip and RHD BeadChip (Immucor). Complex samples that cannot be resolved are referred to CBS. These services are available to all hospitals in the region served by the Ottawa Hospital.

INDICATIONS

For patients in whom genotyping is being considered, a full phenotype should be performed first whenever possible. Because genotyping is complimentary to serologic phenotyping, all genotyping results should have the predicted phenotype confirmed serologically.

Genotyping Should be Considered	
Sickle cell disease (SCD) patients	The American Society of Hematology and the Canadian Hemoglobinopathy Association (CanHaem) SCD guidelines recommend genotyping over phenotyping given the superior detection of partial DCE antigens and delineation of GATA box variants which are critical in this patient population. [5,6]
Complicated antibody investigations	Examples include patients with suspected antibodies against high prevalence or low prevalence antigens, where absence of the antigen from the patient's red cells cannot be confirmed owing to lack of commercially available antisera. Genotyping also may be helpful in distinguishing between an antibody against a high prevalence antigen and an autoantibody in the recently RBC transfused patient.
Prenatal patients	Refer to the NAC Statement on RHD Genotyping in Prenatal Patients.[7] Genotyping may also be useful in the context of complicated antibody investigations (for example, "private antibodies" i.e., reactivity to paternal red cells but without known specificity).
Fetal patients	Complicated prenatal antibody investigations may benefit from fetal and/or paternal genotyping.
Genotyping May be Helpful	
Thalassemia and other hemoglobinopathies where phenotyping is not available/practical	Examples include hemoglobinopathy patients who cannot be phenotyped owing to recent RBC transfusion.
Patients in whom alloantibodies cannot be excluded and phenotyping is not available/practical	Genotyping may be useful in non-phenotyped patients: 1) Receiving monoclonal antibodies such as daratumumab or isatuximab who demonstrate panreactive screens/panels and in whom treatment of reagent red cells with DTT is unsuccessful or not feasible. 2) With autoantibodies in whom advanced techniques (autoadsorption/alloadsorption) are not feasible or are unsuccessful.
Patients at increased risk of forming RBC alloantibodies	Examples include patients receiving chronic RBC transfusion and patients who have demonstrated a marked propensity to

and phenotyping is not available/practical	form RBC alloantibodies. In these patients, provision of antigen-matched RBC units may be used to prevent (further) alloimmunization and genotyping results may facilitate unit selection. Genotyping may also be helpful to resolve serologic discrepancies (most often observed in the context of the D antigen), and thus prevent alloimmunization (please refer to the NAC Statement on RHD Genotyping in Prenatal Patients[7] for more information).
Patients undergoing allogeneic stem cell transplantation who have pre-existing antibodies	Genotyping may be considered for the donor in addition to the patient on a case-by-case basis.
Genotyping is Not Recommended	
Uncomplicated antibody investigation	For example, the presence of an antibody/antibodies where antisera or serologic techniques for the corresponding antigen is commonly commercially available.
Post hematopoetic stem cell transplantation without full engraftment	Current national technologies are not quantitative, and cannot differentiate donor versus recipient-derived antigens, leading to unreliable false heterozygosity of results.

REFERENCES

1. Tormey CA, Fisk J, Stack G. Red blood cell alloantibody frequency, specificity, and properties in a population of male military veterans. Transfusion. 2008 October; 48(10):2069-76. Available from: https://doi.org/10.1111/j.1537-2995.2008.01815.x
2. Telen MJ, Afenyi-Annan A, Garrett ME, et al. Alloimmunization in sickle cell disease: changing antibody specificities and association with chronic pain and decreased survival. Transfusion. 2015 June; 55(6 Pt2):1378-87. Available from: https://doi.org/10.1111/trf.12940
3. Flegel WA, Gottschall JL, Denomme GA. Implementing mass-scale red cell genotyping at a blood center. Transfusion. 2015 November; 55(11):2610–2615. Available from: https://doi.org/10.1111/trf.13168
4. Casas J, Friedman DF, Jackson T, Vege S, Westhoff CM, Chou, ST. Changing practice: red blood cell typing by molecular methods for patients with sickle cell disease. Transfusion. 2015 January 09; 55(6 Pt2):1388-93. Available from: https://doi.org/10.1111/trf.12987
5. Chou ST, Alsawas M, Fasano RM, et al. American Society of Hematology 2020 guidelines for sickle cell disease: transfusion support. Blood Advances. 2020 January 28; 4(2):327-355. Available from: https://doi.org/10.1182/bloodadvances.2019001143
6. The Canadian Haemoglobinopathy Association. Transfusion. In: Consensus Statement on the Care of Patients with Sickle Cell Disease in Canada [Internet]. Ottawa; 2015. [cited February 2024]. Available from: https://www.canhaem.org/wp-content/uploads/2018/05/Sickle-Cell-Consensus.pdf
7. Pavenski K, Khandelwal A, Clarke G, Lane D. NAC Statement on RHD Genotyping in Prenatal Patients [Internet]. Ottawa: National Advisory Committee on Blood and Blood Products; 2022 May 24 [updated 2022 03 15; cited 2024 01 17]. Available from: https://nacblood.ca/en/resource/rhd-genotyping-prenatal-patients
8. Association for the Advancement of Blood & Biotherapies. Technical Manual: Methods and Appendices [Internet]. 20th Edition. Bethesda: Association for the Advancement of Blood & Biotherapies; 2020 [cited November 2023]. Available from: technical-manual-20th-edition-methods-and-appendices.docx (live.com)