

Chapter 6: Donor Selection, Donor Testing and Pathogen Reduction

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BACKGROUND

All blood transfused in Canada is collected from volunteer donors. To ensure the safety of blood components, donors are carefully screened. In addition, the donated blood is tested to identify blood group, blood group antibodies and transfusion-transmissible pathogens. Donor eligibility criteria and testing also benefit the donors by reducing potential blood donation-related health risks.

This chapter describes the donor selection process, the testing done on donated blood, and the pathogen inactivation processes that can further reduce risk of transfusion-transmitted diseases. This chapter is complemented by [Chapter 7](#), Fractionated Blood products and Associated Pathogen Safety, and [Chapter 8](#), Pre-transfusion Testing, of this *Guide*.

DONOR SELECTION

Donors are questioned about medical conditions and behaviours to determine if their blood donation would pose an increased risk to their own health or the health of the recipient.^{1,3}

At each donation, a donor must present identification. At the time of booking an appointment, any pre-existing deferral code would only permit a booking after the deferral date has expired or if the deferral is permanent, the booking would be prohibited. Currently, donations are by appointment only.

Prior to each donation, donors are asked to read a pamphlet, [What you must know to give blood](#), explaining the donation process, the testing that will be done on their blood and the provincial requirements for reporting certain test results to public health authorities. The pamphlet also explains the transfusion-transmission risk factors for human immunodeficiency virus (HIV) and hepatitis and informs donors that testing may fail to identify individuals who are in the early stages of infection.

After reading the pamphlet, donors must complete a standard electronic questionnaire on the day of donation. Donors may fill out the questionnaire at home or in the clinic.

Donors must be at least 17 years old (18 years old at Héma-Québec) and meet height and weight requirements. In donors under the age 23, the donor's sex, height and weight measurements are used to estimate their total blood volume (EBV). An EBV below 3.5 L is associated with an increased risk of fainting from blood donation. Hence, younger first-time donors below an EBV of 3.5 L are deferred.

Donors' medical history is also assessed. Questions are asked about illness in major organ systems that may put them at increased risk of an adverse reaction to donation. Donors are also asked about risk factors for transfusion-transmissible diseases. While there are laboratory tests performed on every donation to detect specific agents (see section below), currently, the questionnaire is the only means of excluding donors with a risk of Creutzfeldt-Jakob disease (CJD), variant CJD, Ebola virus, malaria, Zika virus, babesiosis, leishmaniasis or COVID-19 (viral agent SARS-CoV-2). Donors are not tested for these agents. Donors are therefore asked about travel outside of Canada and deferred for a specific time period depending on the location of travel and their length of stay abroad. For instance, travellers to a region where malaria is considered endemic are deferred from donation of cellular blood components for three months. Although there is no evidence for transfusion transmission of SARS-CoV-2, there is currently a 14-day deferral for international travel to reduce the risk of

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transmission of COVID-19 within a donation site. Deferral criteria for travel is continually evaluated based on scientific data about emerging pathogens. The latest information on blood donor deferrals related to travel can be found under the [ABCs of eligibility](#) on blood.ca. Depending on the magnitude of the risk, donors may be deferred temporarily or indefinitely. For example, currently, people who have identified as having hepatitis are indefinitely deferred, while individuals taking antibiotics may be temporarily deferred until they have recovered completely and no longer require treatment.

Currently, it is estimated that less than four in 100 eligible Canadians donate blood each year. The average donor donates slightly less than twice a year. Approximately 9% of donors are deferred based on the donor questionnaire; most of these are temporary deferrals.

BLOOD COLLECTION PROCESS

Whole Blood Donations

Prior to donation, the donor's temperature is taken and must be below 37.5 °C. In addition, a screening test for hemoglobin level is done on a capillary blood sample. Both arms are examined for signs of injection intravenous drug use. The donor's skin is disinfected using a swab stick containing 2% chlorhexidine gluconate and 70% isopropyl alcohol. An alternative disinfection protocol is used for donors allergic to chlorhexidine. For more information read the Canadian Blood Services publication, "[Alternative methods of blood donor skin disinfection.](#)"

Approximately 6% of whole blood donors are deferred due to anemia. Blood donation, through loss of iron in the red blood cells, can cause iron deficiency anemia. Based on studies of iron deficiency in blood donors, Canadian Blood Services revised its whole blood donor eligibility criteria in 2017⁴ by increasing the donation interval for female donors to 84 days (from 56 days), maximum 4 times a year; the minimum inter-donation interval for male donors remains 56 days (maximum 7 times a year). The minimum hemoglobin level for male donors was increased to 130 g/L; while the minimum level remained at 125 g/L for female donors. For more information, see the Canadian Blood Services publication, "[The importance of iron for whole blood donors: a Canadian perspective.](#)"

Phlebotomy is performed using a sterile single-use kit that contains an anticoagulant nutritive solution. The time for phlebotomy varies from 10 to 15 minutes. Approximately 480 ml of blood are collected per donation. The first few millilitres of blood are directed to a diversion pouch before the main collection bag is filled. Use of a diversion pouch has been shown to decrease contamination of the collection bag with bacterial skin flora. The blood in the diversion pouch is used for the serological and infectious disease testing performed on each unit of blood collected.

Apheresis Donations

The process for screening apheresis donations is very similar to the one for whole blood donation. Several additional criteria are considered to ensure the safety of the donor and the quality of the blood component.

Plateletpheresis donors must have a platelet count of $150 \times 10^9/L$ prior to undergoing each donation. Plateletpheresis may be performed every 14 days for a maximum of 24 donations in a calendar year. Depending on donor characteristics, such as initial platelet count and blood volume, each plateletpheresis donation may yield single or multiple platelet units, or both platelet and plasma components.

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Plasmapheresis donors must have a total serum protein of over 60 g/L and a normal serum protein composition, verified periodically. Donors may make weekly plasmapheresis donations. The maximum quantity of plasma that may be collected per donation and during a twelve-month period can be determined by using either the donor's weight or EBV.

SCREENING TESTS PERFORMED ON BLOOD DONATIONS

Transfusion-Transmissible Disease Testing

Multiple screening tests are performed on each donation to detect the presence of transfusion-transmissible infectious agents. The screening tests performed may detect antigens, antibodies or nucleic acids of the infectious agents. Table 1 provides the screening and confirmatory tests performed at Canadian Blood Services. As formal viral taxonomic nomenclature is constantly changing, common names for viral pathogens will be used in this document.⁵ More information on viral taxonomy can be found on the [International Committee on Taxonomy of Viruses](#) website.

Antibody and antigen tests are done on individual donor samples while nucleic acid testing (NAT) is primarily done on pools of six samples.^{6, 7} The multiplex assay used for NAT enables the simultaneous detection of HIV RNA, hepatitis C virus (HCV) RNA and hepatitis B virus (HBV) DNA. West Nile Virus (WNV) RNA testing is also done in pools of six samples. However, to enhance sensitivity, single unit WNV NAT may be used in selected geographic areas during outbreaks of WNV. Apart from detecting both lineages of WNV, the WNV assay will also react with other viruses in the Japanese encephalitis virus serocomplex including Japanese encephalitis virus (natural infection and vaccination), St. Louis encephalitis virus, Murray Valley encephalitis virus, and Kunjin virus.⁸

NAT for HIV, HCV and HBV are performed on all blood donations throughout the year. Testing is performed for West Nile Virus (WNV) in six-month cycles. Universal donor WNV testing starts on the Monday before June 1st and continues until the last Sunday before December 1; during the rest of the year, selective testing is done on donations from donors identified as being at risk based on the questionnaire due to travel outside of Canada in the eight weeks prior to the donation.

For NAT multiplex screen testing, a reactive pool is resolved to an individual reactive specimen and the viral target that it is reactive for (HIV RNA, HCV RNA or HBV DNA). For WNV the reactive pool is resolved to the individual reactive specimen. Donors reactive on the multiplex screen test are indefinitely deferred, whereas donors reactive on the WNV screen test are deferred for 56 days.

Testing for antibodies to *Trypanosoma cruzi* (Chagas disease) is performed on at-risk donors based on the donor questionnaire. Donors are designated as at-risk if they, their mother or their maternal grandmother were born in Mexico, Central America or South America or if the donor has resided in these countries for a significant period of time.^{9, 10}

Testing for antibodies to cytomegalovirus (CMV) is performed on a small subset of donations to provide CMV-negative components for fetuses receiving intrauterine transfusions.¹¹ Since CMV is a leukocyte-associated virus and all cellular blood components are leukoreduced in Canada, the risk of transfusion-related CMV infection is extremely low. For more information, see [Chapter 15](#), Irradiated, Washed, and CMV Seronegative Blood Components, of this *Guide*.

In April 2021, the Canadian Blood Services serology testing platform and assays for anti-HIV-1/2, anti-HBV, anti-HCV, anti-human T-cell lymphotropic viruses-I/II (HLTV-I/II), and anti-*T. cruzi* changed to a new

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ElectroChemiLuminescence (ECL) assay format. The serologic screening for HIV will move from a 3rd generation anti-HIV-1/2 assay to a 4th generation combination HIV DUO (anti-HIV-1/2 and HIV-1 p24 ECL assay) as well. In the new ECL assay format, there is a very low theoretical risk of high oral doses of biotin (i.e., > 5 mg/day; >125 times adequate use) consumed within 8 hours prior to donation interfering with serology results (e.g., HIV DUO (false negative), anti-HCV (false-negative), anti-HTLV-I/II (false-negative), anti-*T. cruzi* (false-negative), anti-HBV core total (false-positive)). The HBV surface antigen assay has a higher level of biotin-tolerance. The risk of misclassifying a test result due to biotin interference is approximately 1,000–10,000 times lower when compared to other sources of potential interference, with a probability of $\leq 1:10,000,000$ tests. These biotin misclassification probabilities will move closer to zero as biotin tolerances of the other specific assays are increased between 2021 and mid-2023. Since an initial survey indicated that the majority of donors did not know if they consumed higher levels of biotin, donors will not be asked a biotin use question.

Donors who have initially reactive results on antigen or antibody testing have repeat testing performed twice on the same sample. If one of these two repeats is reactive, the donation is discarded, and if the corresponding NAT is negative, additional confirmatory testing is performed for donor notification and counselling. In some instances, if the test result was a false-positive, donors may be invited back for re-testing. Donors may resume donating if repeat test results at least six months post-donation are negative (donor re-entry). Depending on the viral marker, inventory retrieval of blood components from previous donations and notification of the hospitals that received blood components from previous donations (lookback process) may be performed.¹² For more information on this process see [Chapter 1, Vein to Vein: A Summary of the Blood System in Canada](#), of this Guide.

Table 1: Transfusion-transmissible disease testing at Canadian Blood Services.

Pathogens	Screen Tests	Confirmatory/Supplemental Tests
HIV 1/2 (Human immunodeficiency virus, types 1 and 2)	<ul style="list-style-type: none"> • Combination Anti-HIV-1/2 and HIV-1 p24 (4th generation) ElectroChemiLuminescence assay • HIV NAT 	<ul style="list-style-type: none"> • Geenius™ HIV-1/2 confirmatory testing
HBV (Hepatitis B virus)	<ul style="list-style-type: none"> • Hepatitis B surface antigen (HBsAg) ElectroChemiLuminescence assay • Antibody to hepatitis B core antigen (HBcore) ElectroChemiLuminescence assay • HBV NAT 	<ul style="list-style-type: none"> • HBsAg confirmatory testing (ElectroChemiLuminescence assay) • There is no confirmatory testing for HBcore
HCV (Hepatitis C virus)	<ul style="list-style-type: none"> • Anti-HCV ElectroChemiLuminescence assay • HCV NAT 	<ul style="list-style-type: none"> • HCV Line testing (LIA)
HTLV-I/II (Human T-cell lymphotropic viruses, types I and II)	<ul style="list-style-type: none"> • Anti-HTLV I/II ElectroChemiLuminescence assay 	<ul style="list-style-type: none"> • HTLV Western Blot Assay testing

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Pathogens	Screen Tests	Confirmatory/Supplemental Tests
<i>Treponema pallidum</i> (pathogen for syphilis)	<ul style="list-style-type: none"> • Micro-hemagglutination assay for <i>Treponema pallidum</i> (MHATP)* 	<ul style="list-style-type: none"> • Algorithms may vary depending on reference laboratory site and initial/follow-up results • <i>Treponema pallidum</i> Particle Agglutination (TPPA) Test • Rapid Plasma Reagin (RPR) • Fluorescent treponema antibody absorption (FTA-ABS)
WNV (West Nile virus) †	<ul style="list-style-type: none"> • WNV NAT 	<ul style="list-style-type: none"> • Sequencing or alternate NAT may be done at reference laboratory on WNV NAT-positive specimens when history suggests a potential exposure to another member of Japanese encephalitis virus serocomplex
<i>Trypanosoma cruzi</i> (pathogen for Chagas)†	<ul style="list-style-type: none"> • Anti-<i>Trypanosoma cruzi</i> ElectroChemiLuminescence assay 	<ul style="list-style-type: none"> • Enzyme-linked immunosorbent assay (ELISA) • Immunoblot • Polymerase chain reaction (PCR)
CMV (cytomegalovirus) ‡	<ul style="list-style-type: none"> • Anti-CMV particle agglutination assay 	<ul style="list-style-type: none"> • None available

* chicken erythrocytes which have been fixed and then sensitized with components of sonicated *T. pallidum* (Nichols strain)

† performed on at-risk donors

‡ performed on a subset of donations for components required for intrauterine transfusion

Bacterial Testing

Platelets can be stored at room temperature with gentle agitation for up to seven days prior to transfusion. This storage requirement makes platelet units the blood component most likely to be associated with bacterial growth.¹³ All platelet units are tested for bacterial contamination using an automated blood culture system incubated for up to seven days after inoculation. Inoculation is performed at least 36 hours after blood/platelet collection to allow potentially contaminating bacteria a longer period of time to proliferate, thus improving the ability to detect them. See the “[FAQ: Canadian Blood Service Platelet Bacterial Testing](#)” for more about platelet testing. Platelets are issued to hospitals as “negative to date,” and if the culture subsequently becomes positive, will be recalled, along with other blood components from the same donation.

Blood Group Determination and Antibody Detection

ABO, RhD and K (Kell) typing are performed using an automated hemagglutination assay. Confirmatory typing is done for first-time donors found to be Rh negative, and donors are tested for the presence of Rh D and weak D antigens. Canadian Blood Services implemented K testing on all donations in November 2018 and by the end of March 2020 more than 90% of active donors had been phenotyped. The Kell phenotype appears on the unit end label if the donor is antigen negative. Testing is also performed for unexpected red blood cell antibodies by doing an antibody screen on each donation.

The methods used for donor testing may be less sensitive than those required in pre-transfusion antibody detection for recipients. In recipient testing, a low level of antibody may be of clinical importance, since an anamnestic response may occur. In donor testing, only a small amount of passive antibody transfusion will

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occur and is generally clinically insignificant for red blood cell transfusion. For more information read the Canadian Blood Services article, "[Whole blood donors with antibodies](#)." Plasma and platelets are not produced from donations containing red blood cell alloantibodies.

Phenotyping for RhC/c, RhE/e, Duffy, Kidd and S/s is routinely performed on a subset of donor samples using an automated, algorithmic approach. This has led to a substantial inventory of red blood cell units end-labeled as negative for those antigens. In general, approximately 40% of donors have been phenotyped for the common clinically significant antigens. Red blood cell antigen genotyping is also performed on some donors. This testing allows for identification of certain rare phenotypes and confirms variable serologic reactivity for some antigens. Genotype testing may also be performed when serologic reagents are not readily available to define a particular red blood cell antigen type or when family members of rare donors are investigated as part of the Canadian Blood Services [rare blood program](#). Negative phenotypes identified by serology or genotyping are reported on the unit end label. See Canadian Blood Services' [serological best practices](#) resources and publication on [phenotype matching for sickle cell patients](#) for more information on how to use antigen typed blood components in transfusion practice.

Platelet components can be tested for human leukocyte antigen (HLA) or human platelet antigens (HPA) to manage patients who, for example, have documented antibodies targeting an HPA or alloimmune refractoriness. For more information about platelet components testing and utilization see [Chapter 12](#), Hemolytic Disease of the Fetus and Newborn and Perinatal Immune Thrombocytopenia, and [Chapter 18](#), Platelet Transfusion, Alloimmunization, and Management of Platelet Refractoriness, of this *Guide*.

PATHOGEN INACTIVATION SYSTEMS

Current pathogen inactivation (PI) technologies are based on chemical or photochemical principles that target and damage nucleic acids (disrupt DNA and RNA). They significantly limit replication and growth of pathogens such as bacteria, viruses and protozoa thereby preventing infectivity.^{14, 15} Applications of these technologies for the treatment of red blood cell components and for units of whole blood are under development in clinical trials.^{14, 16}

Pathogen inactivation methods for plasma components include solvent-detergent treatment of plasma in pools and methylene blue followed by ultraviolet A (UVA) illumination for single unit plasma.¹⁷⁻¹⁹ Both these methods are in use in several European and other jurisdictions.^{20, 21}

Pathogen inactivation methods for both plasma and platelets components include amotosalen S59 (a psoralen) plus UVA illumination, riboflavin plus UVA/B light illumination, and UVC illumination alone.^{14, 22} The first two methods are in use for plasma and/or platelets components in Europe, Asia, the Middle East, Central and South America. Amotosalen plus UVA illumination was approved for apheresis platelets in the United States in 2015 and in Canada in 2018. Riboflavin plus UVA/B light illumination for platelet components was tested in an international clinical trial with Canadian Blood Services participation and should be licensed in Canada shortly.^{23, 24}

Amotosalen plus UV illumination was licensed for platelet components in Canada in 2018. A pooled plasma component that has undergone PI using solvent-detergent is licensed and is available with some restrictions in Canada.

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CONTINUING PROFESSIONAL DEVELOPMENT CREDITS

Fellows and health-care professionals who participate in the Canadian Royal College's Maintenance of Certification (MOC) Program can claim the reading of the Clinical Guide to Transfusion as a continuing professional development (CPD) activity under Section 2: Self-learning credit. The reading of one chapter is equivalent to two credits.

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