Chapter 15: CMV Seronegative, Irradiated and Washed Blood Components

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BACKGROUND

Modified or specialty blood components may be useful in specific clinical settings to reduce the risk of transfusion-related harm. Specialty blood products available to physicians include CMV-seronegative, irradiated, and washed red blood cells or platelets. This chapter describes the preparation of these blood components and the clinical setting in which they are of greatest benefit.

CMV-SAFE COMPONENTS TO PREVENT TRANSFUSION-TRANSMITTED CMV INFECTION

Characteristics of CMV infection

Cytomegalovirus (CMV), also known as human herpesvirus-5, is a large, enveloped, double-stranded DNA virus. Primary CMV infection can occur following contact with body fluids (e.g. saliva, breastmilk or urine) of an individual actively shedding viral DNA, or following blood transfusion (transfusion-transmitted CMV infection; TT-CMV) or transplantation of organs and tissues from donors known to have previously been infected with CMV. In a healthy individual, primary CMV infection is often asymptomatic or results in a mild, non-specific illness similar to mononucleosis. However, in immunocompromised individuals, CMV infection can result in CMV disease, which can include life-threatening pneumonia, hepatitis and colitis. Primary CMV infection in an expectant mother may, in rare cases, lead to congenital CMV in the newborn infant.

Individuals who recover from a primary CMV infection develop an immune response and become CMV IgG antibody positive, or CMV-seropositive, approximately six to eight weeks after contracting the virus. During this window period of seroconversion, viral DNA can rarely be detected in the plasma. The virus then becomes dormant (latent) in the monocyte population of white blood cells and may reactivate at a later time. Therefore, CMV virus is never “cleared” from the body. The prevalence of CMV-seropositive individuals is estimated to be 53% in the Canadian population, and ranges from 20% to 100% in adults world-wide. The seroconversion rate among Canadian Blood Services’ donors is approximately 0.7% annually. Blood donation during the window period is rare.

CMV reduction strategies during the preparation of blood components

In Canada, CMV serologic screening of blood donors was implemented in the mid-1980s. Canadian Blood Services introduced universal leukoreduction (LR) in 1999. This process decreases the leukocyte (white cell) concentration of cellular blood components from approximately 1 x 10^8 to less than 5 x 10^6 leukocytes per unit, removing a significant proportion of CMV-containing cells. Although LR substantially reduces the risk of TT-CMV, it cannot be completely eliminated.

Hence, all cellular components that are LR are considered “CMV-safe”.

Red blood cells and platelets produced by the buffy coat method are rendered CMV-safe by leukofiltration during component processing. Platelets produced by apheresis are leukoreduced during the apheresis procedure. Plasma is relatively acellular as a result of the whole blood processing and plasma freezing methods, and is considered CMV-safe without undergoing additional filtration, with no apparent risk of CMV transmission.

None of the present CMV reduction strategies remove CMV DNA or virions found in donor plasma, such as during the window period when CMV serology is negative, or during CMV reactivation. Additional CMV serologic testing does not guarantee that a CMV seronegative component will truly be “CMV negative”. However, the

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Published: Friday, August 4, 2017

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concentration of CMV DNA required to cause TT-CMV infection is unknown. CMV DNA has been detected in 0.04% of units labelled as "CMV-seronegative", with recent evidence suggesting that the greatest concentration of CMV DNA is present in the plasma within the first year of seroconversion following primary infection. Nucleic acid testing for CMV DNA is not presently performed by Canadian Blood Services.

Clinical evidence supporting best practices for the prevention of TT-CMV

The only Canadian Consensus Conference on the prevention of post-transfusion CMV to date was held in January 2000, following which a consensus statement was published based on available literature and expert opinion. The majority view of the conference panel recommended continued CMV serologic screening of donors and provision of CMV-seronegative components following implementation of LR in Canada, with the rationale that a combined approach could be additive in its efficacy for prevention of TT-CMV (the “belt and suspenders approach”). According to the consensus statement, transfusion of CMV-seronegative/LR components was recommended for patient groups at highest risk for TT-CMV, including: intrauterine transfusions, CMV-seronegative pregnant women, and CMV-seronegative recipients of allogeneic bone marrow transplantation.

The prevalence of TT-CMV was reported to be 10–60% before CMV serologic screening and LR were adopted, and was typically seen three to eight weeks after transfusion of an infected cellular component. Contemporary strategies of reducing TT-CMV include provision of cellular components which are CMV-seronegative or LR. Based on test sensitivity and leukoreduction efficiency, each method was found to have an estimated residual risk of CMV transmission of approximately 1.5–3% per transfused recipient. However, in a recent Australian study, the residual risk of TT-CMV from LR red blood cells and platelets (combined) was shown to be much lower: 1 in 13.5 million units transfused. In Canada, the residual risk of TT-CMV is also very low at 1 in 680,000 red blood cell units transfused and 1 in 186,000 platelet units transfused.

Observational studies in allogeneic hematopoietic stem cell transplant (HSCT) patients have not demonstrated any increased risk of TT-CMV with LR/CMV-untested (CMV-safe) cellular components. In practice, the risk of CMV reactivation and primary infection from the environment in the immediate post-HSCT period has led to routine monitoring of recipients for CMV viremia, with initiation of pre-emptive treatment if increasing CMV copies are detected. Studies comparing TT-CMV risk of LR components with CMV-seronegative/LR components in intrauterine transfusion and low birthweight infants have not been published. However, transmission of CMV in breastmilk appears to be the greatest risk factor for CMV disease in low birthweight infants.

Provision of CMV-seronegative/LR blood products varies among major transfusion centers internationally, as demonstrated by a survey published in 2014. The population size needed to perform an adequately powered study to determine whether a combined CMV-seronegative/LR strategy is equivalent to either modality alone is prohibitive, meaning this study will likely never be performed. A recently published systematic review could not favor a specific strategy (single or combined CMV reduction modality) for TT-CMV reduction based on available scientific evidence. As a result, the AABB CMV Prevention Working Group could not make a recommendation regarding the appropriate usage of LR and/or CMV-seronegative blood components.

According to the Canadian Standards Association Z902-15, Blood and Blood Components, standard 11.6, each transfusion service should have a written policy indicating which recipients or categories of recipients are to receive cellular blood components selected or processed to reduce the risk of CMV transmission.

The Canadian National Advisory Committee on Blood and Blood Products (NAC), which is comprised of national transfusion medicine experts and has a mandate to provide advice on blood utilization management and transfusion medicine practice, includes a CMV subcommittee which has recently evaluated available literature on reduction of TT-CMV in the post-LR era. Upon the recommendation of the CMV subcommittee, NAC members (representing all provinces, except Québec) have agreed that CMV-safe (LR) cellular blood products are

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**equivalent in safety to CMV-seronegative/LR blood products for transfusion in all patient populations except for intrauterine transfusion.** Online publication of this consensus statement and references leading to this decision are posted on the NAC website.

**NAC’s statement regarding appropriateness of use of Cytomegalovirus (CMV) sero negative vs. CMV safe product**

**Recommendation #1:** The National Advisory Committee recommends that CMV safe (leukoreduced) and CMV IgG seronegative products be considered equivalent except for Intrauterine transfusion.

**Recommendation #2:** The National Advisory Committee recommends that Canadian Blood Services stop their current process for testing and provision of CMV seronegative units issued to hospital facilities and develop a new process to maintain a small inventory of CMV seronegative blood components for the sole purpose of Intrauterine transfusion.

**Recommendation #3:** The National Advisory Committee recommends that Canadian Blood Services explores the feasibility of providing a small boutique inventory of dually tested (seronegative and NAT) CMV negative blood components for the sole purpose of Intrauterine transfusion.

**IRRADIATED BLOOD COMPONENTS TO PREVENT TRANSFUSION-ASSOCIATED GRAFT VERSUS HOST DISEASE**

Characteristics of transfusion-associated graft versus host disease

Transfusion-associated graft versus host disease (TA-GvHD) is a consequence of transfusion of cellular blood components containing viable T-lymphocytes into a recipient whose immune system is not capable of eliminating these cells. This occurs because the host immune system is either weakened, or does not recognize the infused cells as foreign. When the recipient fails to eliminate the donor lymphocytes, they proliferate and attack recipient tissues. Two populations identified to be at especially high risk of TA-GvHD include: 1) recipients of directed donations from family members or HLA-matched platelet transfusions, when there are recipient-donor Human Leukocyte Antigen (HLA) similarities or 2) immunocompromised recipients.

TA-GvHD is a rare but serious transfusion complication with a mortality rate over 90%. Symptoms include fever, maculopapular or erythematous rash, diarrhea, hepatitis and progressive bone marrow failure. Initial signs may appear eight to ten days post-transfusion, and progress to irreversible pancytopenia three to four weeks post-transfusion. Death from bleeding or infection often occurs one to three weeks after initial symptom onset. Diagnosis is based on characteristic pathologic changes on skin biopsy and/or demonstration of donor lymphocytes in recipient tissues using molecular, cytogenetic or tissue typing techniques. Treatment is supportive, and cure has only been rarely reported after rapid hematopoietic stem cell transplantation.

**TA-GvHD reduction strategy during the preparation of blood products**

Prevention is the key to reducing mortality related to TA-GvHD. Irradiation of cellular blood products using a gamma-ray source (Cesium-137 (Gammacell 1000) or Cobalt-60), or X-ray source (Raycell) is the only effective technique for the prevention of TA-GvHD. Irradiation inflicts irreparable DNA damage to the T-lymphocytes and prevents them from replicating, thereby preventing TA-GvHD.

The recommended radiation dose is 25 cGy to the central point of the blood pack with a minimum dose of 15...
cGy to other parts, and a maximum dose of 50 cGy. All parts of the pack must be irradiated, as even a small amount of lymphocytes found in peripheral tubing may cause TA-GvHD. Time for component irradiation is dependent on the radiation intensity of the source. Commercially available indicator labels are applied to the blood product to verify that an adequate radiation dose has been delivered.

### Clinical practice

LR alone is an insufficient prevention strategy as TA-GvHD has been reported following the transfusion of LR blood components. The minimum lymphocyte dose capable of causing TA-GvHD is unknown. Risk likely depends more on the degree of HLA similarity between the blood donor and the transfusion recipient than on degree of immunocompromise, as highlighted by a recent systematic review which showed that the majority of reported TA-GvHD occurred in immunocompetent hosts.

Cellular blood products must be irradiated prior to transfusion to patients in specified risk groups (Table 1). Other blood products that are frozen without cryoprotective agents (fresh frozen plasma (FFP)/frozen plasma (FP), cryoprecipitate and cryosupernatant plasma) and fractionated plasma products have not been associated with TA-GvHD and do not require irradiation. The British guidelines on irradiated blood components note that no cases of TA-GvHD have been reported after transfusion of cryopreserved red blood cells, and do not recommend irradiation for this component. Canadian Standards Association Z902-15 for blood and blood components does not comment on the requirement for irradiation of cryopreserved red blood cells.

#### Table 1: Cellular blood products requiring irradiation

<table>
<thead>
<tr>
<th>Blood products to be irradiated prior to transfusion to patients at risk for TA-GvHD</th>
<th>Blood products to be irradiated prior to the transfusion to any patient*</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Whole blood</td>
<td>• Directed donation of cellular components from a related family member</td>
</tr>
<tr>
<td>• Washed red cells</td>
<td>• Granulocyte concentrates*</td>
</tr>
<tr>
<td>• Platelets (prepared from whole blood or from apheresis collection)</td>
<td>• HLA-matched platelets</td>
</tr>
</tbody>
</table>

* Canadian Blood Services does not provide granulocyte concentrates, but they are available from Héma-Québec.

Irradiation of cellular blood products is considered essential for patients with a well-defined risk for TA-GvHD; however, this requirement is debatable for patients with an identified but not clearly defined risk (Table 2).

Irradiation of cellular blood products is not considered necessary for patients with HIV infection, recipients of solid organ transplants, or patients with isolated humoral immune deficiency disorders such as hypogammaglobulinemia. Most cases of TA-GvHD reported in infants occurred when intrauterine transfusion was followed by exchange transfusion.

#### Table 2: Patients at risk for transfusion-associated graft versus host disease (TA-GvHD)


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Patient groups with a well-defined risk for TA-GvHD

- Fetuses undergoing intrauterine transfusion
- Newborns who have previously undergone intrauterine transfusions, until after the expected delivery date (40 weeks gestational age)
- Newborns undergoing exchange transfusion
- Neonates with a complex cardiac abnormality, until a congenital immunodeficiency is ruled out
- Patients with congenital T-cell immunodeficiency, including:
  - Severe combined immunodeficiency disease (SCID)
  - Di George syndrome
  - Wiskott-Aldrich syndrome
  - Purine nucleoside phosphorylase deficiency
  - Reticular dysgenesis
  - Cell-mediated immune deficiency of unspecified etiology
- Selected patients with acquired immunodeficiency:
  - Hodgkin lymphoma
  - Receiving or having received treatment with purine antagonists (fludarabine, cladribine, deoxycofomicin); or purine-like antagonists (bendamustine, clofarabine);
  - Receiving or having received treatment with potent T-cell inhibitors alemtuzumab (anti-CD52) therapy for any indication; or anti-thymocyte globulin (ATG) for severe aplastic anemia
- Hematopoietic stem cell transplant recipients:
  - Allogeneic transplant – for life
  - Autologous transplant – 3 months following transplant if no total-body irradiation conditioning; 6 months following transplant with total-body irradiation conditioning

Patient groups with an identified but not clearly defined risk for TA-GvHD

- Preterm infants with low birth weight requiring small volume (top-up) transfusion
- Patients with hematologic malignancies other than those listed above

Irradiation irreparably damages the red blood cell membrane, increasing the rate of potassium loss and precipitating hemolysis with decreased red blood cell recovery. As a result, Canadian Blood Services and many Canadian hospitals have voluntarily chosen to follow the Council of Europe Standards, 17th Edition (2013) recommendations, which state that:

Red cell components may be irradiated up to 28 days after collection. Irradiated cells must be transfused as soon as possible, but no later than 14 days after irradiation, and in any case, no later than 28 days after collection.29

For neonatal patients who are particularly susceptible to the effects of hyperkalemia, the freshest red blood cells (maximum seven days from collection) should be chosen for irradiation, and irradiation should occur immediately prior to issue. If the component is not used immediately, its shelf-life is reduced to 14 days from the date of irradiation, not exceeding the original component expiry.

For patients at high risk of complications from hyperkalemia (e.g. pediatric cardiac surgery) or those receiving high-volume transfusions (e.g. intrauterine or exchange), single-washing of red blood cells followed by supernatant removal must be performed to reduce potassium levels if more than 24 hours has elapsed since the

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unit was irradiated. While some differences in quality parameters have been identified between irradiated and non-irradiated platelet products, these findings have not been significant enough to impact storage guidelines. The expiry of platelet units is not impacted by irradiation.

The Irradiation Working Group subcommittee of the Canadian National Advisory Committee on Blood and Blood Products (NAC) is developing recommendations for irradiated component use in Canada. These are expected to be published in the fall of 2017.

**WASHING BLOOD PRODUCTS TO REDUCE RESIDUAL SUBSTANCES**

Cellular blood components may be modified by washing to reduce the level of residual substances (e.g. antibodies, serum proteins such as IgA, additive solutions, potassium, other cellular metabolites or cytokines) that may be harmful for some transfusion recipients. Hospital-based transfusion medicine laboratories which modify or transform cellular components, including washing of red blood cells or platelets, require Health Canada licensure.

**Washed red blood cell products**

Red blood cell washing is a modification applied to a standard red blood cell product that has undergone pre-storage LR by filtration at Canadian Blood Services. The red blood cells are washed several times with compatible solution, most commonly sterile 0.9% sodium chloride injection USP (normal saline), and then resuspended prior to transfusion. The volume of fluid required is dependent on the indication for the washed product, but typically involves 1–3 litres of wash solution. Washing may be performed manually or by an automated procedure using specialized blood processing equipment, and markedly reduces the levels of plasma proteins, antibodies and electrolytes in the component.

Canadian Blood Services is able to provide washed red blood cells upon request. In 2013, Canadian Blood Services implemented a new closed-system (sterile) automated wash procedure with specialized equipment, where red blood cell units within the first two weeks of storage are washed, then resuspended in 100 ml of SAGM additive solution. This method extends the permissible product storage duration to 7 days post-wash. Depending on the indication and wash procedure, the final components are labelled “Red Blood Cells LR, Washed” or “Red Blood Cells LR, Extra Wash (IgA deficient)”. Washing is not available at all Canadian Blood Services manufacturing sites, so additional time for site transfer may be required.

The manual red blood cell wash process is considered an open (non-sterile) procedure, and may be performed at licensed tertiary care hospitals in Canada. A spike is inserted into the bag to facilitate transfer of the red blood cells into a transfer bag, followed by centrifugation (the initial transfer is necessary to avoid bursting of the bag, which is not intended to undergo multiple centrifugation steps). The plasma supernatant is removed, and 200–250 ml of sterile wash solution is then added to rinse the cells in the bag, followed by centrifugation and supernatant reduction. This procedure is repeated a minimum of three times for a manual wash. The non-sterile opening of the red blood cell bag increases the risk of bacterial contamination during processing and reduces the expiry time of the final product to 24 hours from the beginning of the wash procedure. Manual wash of red blood cell units is performed within licensed hospital transfusion medicine laboratories only.

If appropriate equipment is available, automated red blood cell wash procedures may also be performed at the...
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hospital setting. Wash procedures and product expiry timelines are dependent on the equipment manufacturer specifications. In general, automated wash procedures performed at the hospital level are non-sterile procedures with product expiry 24 hours from the beginning of the wash of the LR red blood cell product. Generally, washing by either manual or automated method adds at least two hours to the processing time of each red blood cell unit.

Storage conditions and the administration process for washed red blood cells are the same as those for unwashed red blood cells, with the exception of a reduced expiry time (dependent on “open” or “closed” wash methodology). Viability of washed red blood cells is compromised if the anticoagulant-preservative solution is removed during washing and not replaced. Red blood cell recovery may be decreased by up to 20–25% due to red blood cell loss during washing (greater loss with automated wash).

Risks associated with red blood cell transfusion also apply to the washed product, although the risk of bacterial contamination is slightly higher if an open washing system is used. The incidence of febrile and allergic reactions is reduced due to the removal of white blood cells and plasma from the product.

Historically, patients with documented IgA deficiency were transfused with washed red blood cells due to the concern of an increased risk of anaphylactic reaction with standard red blood cell products. A recent literature review has demonstrated that the incidence of anaphylactic reaction with red blood cell transfusion in patients with true IgA deficiency is approximately equivalent to that of the general population (about 1:50,000 transfusion events). However, if a patient is confirmed to have clinically significant IgA deficiency, the current methodology used by Canadian Blood Services for “extra wash” red blood cells has been validated to provide a product that meets the definition of being IgA deficient, containing less than 0.05 mg/dl of IgA protein.

Current indications for washed red blood cells include:

- IgA deficient patients with a documented anti-IgA, when red blood cells from an IgA deficient donor are unavailable.
- Patients with a history of anaphylactic transfusion reactions of unknown etiology.
- Recurrent and/or severe febrile or allergic transfusion reactions, if not ameliorated by pre-transfusion medications or responsive to plasma reduced red blood cells.

Most hospital-based transfusion medicine services that provide washed red blood cells will also provide a “single-washed” or “additive depleted” red blood cell product, where after adding some normal saline into the red blood cell bag, the unit is then centrifuged to remove the plasma supernatant, and resuspended in a small volume of compatible fluid (5% albumin, 0.9% sodium chloride injection USP, or ABO type compatible frozen plasma as appropriate) prior to transfusion to a desired hematocrit. Similar to manually washed red blood cells, these blood products expire within 24 hours and may be requested:

- Prior to neonatal exchange transfusion or massive transfusion in neonatal/pediatric patients to minimize the amount of additive solution and/or potassium in the component.
- To mitigate recurrent moderate or severe medication-refractory febrile or allergic transfusion reactions by removing potentially responsible plasma proteins or cytokines.

According to the Canadian Standards Association Z902-15 for Blood and Blood Components, standard 7.5.3.2, washed red blood cells shall be prepared by a method known to retain at least 75% of the red cells that were in the original red blood cells and yield a hematocrit of not more than 0.8 L/L.

Washed platelet products

Platelet products may also be washed to remove plasma and supernatant substances such as antibodies or
other serum proteins that may be harmful to some transfusion recipients. Platelets prepared from whole blood donations or harvested by apheresis may be washed using normal saline, or saline buffered with ACD-A or citrate. The washing may be a manual or automated process (dependent on equipment availability).

Washed platelets may be indicated for:

- IgA deficient patients with a documented anti-IgA, when platelets from an IgA deficient donor are unavailable.
- Patients with a history of anaphylactic transfusion reactions of unknown etiology.
- Recurrent and/or severe febrile or allergic transfusion reactions, if not ameliorated by pre-transfusion medications or responsive to plasma reduced platelets.
- Neonates with fetal/neonatal alloimmune thrombocytopenia (FNAIT), if the platelets are acquired from the baby’s mother.

In the clinical setting of FNAIT, if a matching human platelet antigen (HPA)-negative allogeneic volunteer apheresis donor is not available, maternal platelets may be collected and washed to remove HPA antibodies, with subsequent irradiation and directed transfusion into the affected neonate. However, this approach is rarely needed, as allogeneic HPA-matched platelet donors can be found in most circumstances. As there is an increased risk of bacterial contamination and possible metabolic damage to platelets, washed platelets must be administered within four hours of beginning the wash procedure. Since the washing procedure itself takes at least two hours, the timing of the washing procedure with respect to the timing of the transfusion event must be carefully coordinated.

Canadian Blood Services does not currently provide washed platelet components. Therefore, hospitals requiring these products must prepare them in the hospital blood bank. There is considerable (up to 20%) platelet loss as a result of platelet activation during washing. However, it has been demonstrated that the viability and efficacy of remaining platelets is not significantly affected.

Due to the high platelet loss associated with washing, plasma volume reduction (platelet concentration) is considered a more suitable option for those situations in which complete removal of plasma by washing is not required, though platelet loss may still occur with this method due to platelet activation. Plasma reduction of platelets may be used prior to transfusion of ABO-incompatible platelets in a shortage situation to reduce the risk of isohemagglutinin-mediated red blood cell hemolysis in platelet recipients, or to volume-reduce platelets for neonatal recipients with very small blood volumes.

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0-23-eng.php (Last accessed May 2 2016).

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