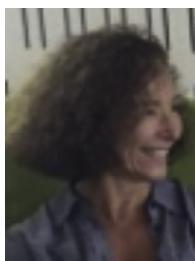




## REVIEW



# Managing and preventing blood-borne viral infection transmission in assisted reproduction: a Canadian Fertility and Andrology Society clinical practice guideline



## BIOGRAPHY

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## KEY MESSAGE

People who are living with HIV or hepatitis and have a viral load that is undetectable or unquantifiable are not infectious and pose no risk of cross-contamination in the ART laboratory. ART laboratories should process these gametes in the usual fashion without any special precautions needed to prevent infection.

## ABSTRACT

Fertility care providers have an obligation to provide safe and effective care to patients. When a user of assisted reproductive technology (ART) is living with a blood-borne viral infection (BBVI: HIV, hepatitis C or hepatitis B), physicians and ART laboratory personnel need to know the requirements for providing quality care. Recent developments in the treatment of BBVI and understanding of transmission have changed these requirements. This guideline from the Canadian Fertility and Andrology Society (CFAS) provides comprehensive, evidence-based guidelines for reducing horizontal transmission and cross-contamination in the ART setting.

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This Clinical Practice Guideline has been prepared by the Canadian Fertility and Andrology Society (CFAS) Clinical Practice Guideline Committee and approved by the CFAS Clinical Practice Guideline Committee<sup>†</sup> and the Board of the CFAS. This document is endorsed by the Association of Medical Microbiology and Infectious Disease Canada (AMMI).

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## INTRODUCTION

**F**ertility care providers have an obligation to provide safe and effective care to patients. When a user of assisted reproductive technology (ART) is living with a blood-borne viral infection (BBVI: human immunodeficiency virus [HIV], hepatitis C virus [HCV] or hepatitis B virus [HBV]), physicians and ART laboratory personnel need to know the requirements for providing quality care.

ART laboratories routinely safeguard against risk of sample mix-up, equipment failure and contamination by other organisms. These processes that are standards of care are also important in the management of BBVI. This guideline provides an evidence-based approach to reduce the risk of BBVI transmission through horizontal transmission or cross-contamination when oocytes, spermatozoa or embryos from a person living with a BBVI are used.

The nature of ART inherently prevents female-to-male transmission, and intrauterine insemination (IUI) can be used to prevent horizontal spread when sexual intercourse is a source of potential infection. When having penile-vaginal intercourse without a condom is considered acceptable with respect to the risk of BBVI transmission in the general population, this guideline recommends no additional processes in the ART laboratory.

Cross-contamination in ART is theoretically possible when a person with a BBVI is being treated. However, the mere presence of measurable viral DNA or RNA is not sufficient to cause infection. The risk of infection is commensurate with cell number or fluid volume. Human blastocysts contain fewer than 200 cells and are situated in less than 0.5 ml of media. Haematological samples, which are the more common sources of infection through cross-contamination, have volumes of 25–75 ml and millions of cells. As a general principle, BBVI require direct contact of blood (or semen) into a recipient. Thus, spread via fomite or environmental surface is not likely. With direct contact (i.e. spillage), a further requirement for infection is the presence of a host cell. The risk of cross-contamination in the incubator is based on the potential infectivity of the gamete, the likelihood of its media coming in direct contact with

another gamete, and the potential of the recipient gamete to become infected.

Using human serum in culture media has been associated with cross-contamination when HBV-infected serum was used, when non-commercial media were made with the addition of patients' serum (*Quint et al., 1994*). Follow-up from such an event did not demonstrate any infection in offspring although there was acute hepatitis in most women affected (*van Os et al., 1991*). There have been no reported cases of BBVI occurring through cross-contamination in the ART laboratory in the two decades since the widespread use of commercial media. There is one report of hepatitis C transmission that occurred through contamination in the venepuncture setting and not through gametes or embryos (*Lesourd et al., 2000*).

The regular emergence of new pathogens underscores the need for constant best practices in infection control. In Canada, infection control guidelines are determined provincially, for example by Public Health Ontario (Ontario Agency for Health Protection and Promotion). When processes are not appropriate for the IVF setting, the specific provincial regulators may provide alternates. Options for sterilization processes for HIV are well described by Public Health authorities, for example Ontario (*Agency for Health Protection and Promotion et al., 2013*).

This guideline does not address BBVI risks associated with (i) tissue (ovarian or testicular) procurement, (ii) perinatal transmission, or (iii) any other pathogens. For recommendations for HIV in pregnancy the Society of Obstetricians and Gynaecologists of Canada's (SOGC) (*Money et al., 2014*), and for hepatitides the Canadian Association for the Study of Liver Disease (CASL) and the Association of Medical Microbiology and Infectious Disease (AMMI), publications on HBV (*Coffin et al., 2018*) and HCV (*Shah et al., 2018*) can provide guidance. Specific considerations for general pregnancy planning are not included in this guideline but can be found in the SOGC's guidelines for those affected by HIV (*Loutfy et al., 2018*).

This document is intended to provide guidance only for autologous samples. The guideline does not pertain to the use of donor gametes. Health Canada

regulations on third-party reproduction supersede this document. The accepted process for screening of and use of directed donors can be found in Health Canada guidance documents (<https://www.canada.ca/en/health-canada/programs/consultation-safety-sperm-ova-regulations/document.html#a12>).

## METHODS: GUIDELINE DEVELOPMENT

This guideline is informed by the available research data on transmission and empirical ART data relevant to managing BBVI in the ART setting. In accordance with Canadian Fertility and Andrology Society (CFAS) requirements, the guideline development working group used the Grading of Recommendations Assessment, Development and Evaluation (GRADE) ('GRADE home,' n.d.) approach to development of recommendations when research was available. This provides a framework for the guideline development process, including assessment of the quality of evidence and recommendations.

### Literature search strategy

In July 2018, literature searches were performed by a librarian to identify issues related to BBVI ART transmission associated with cross-contamination, sperm washing and other ART-related procedures. Databases searched included Ovid MEDLINE, the Cochrane Central Register of Controlled Trials and Embase from the time of inception to the date of completion of searches. For the first search on cross-contamination, databases were searched using terms such as "HIV" or "HBV" or "HCV" and "IUI" or "IVF" or "Cryopreservation". For the second search on sperm washing, databases were searched with terms such as "Sperm" and "Wash" and "HIV" or "HBV" or "HCV". Full search terms can be provided on request. Titles and abstracts were screened for inclusion, followed by full-text review by two independent reviewers; a third reviewer was consulted to resolve discordance. A total of 11 articles for cross-contamination and eight articles for sperm washing were included for the final selection.

## PROVISION OF ART WHEN THERE IS HIV INFECTION

### Background

A total of 2402 new HIV cases were reported in Canada in 2017, a diagnosis

rate of 6.5/100,000 (*Haddad et al., 2019*). The 30–39-year age group represents the highest number of new HIV cases. Although Caucasians accounted for the majority of reported diagnoses in 2017, both Indigenous and Black people were disproportionately represented, each making up less than 5% of the Canadian population but each accounting for more than 20% of new diagnoses (*Centre for Infectious Disease Prevention and Control (Canada) and Public Health Agency of Canada, 2013*).

The introduction of combination antiretroviral therapy in the mid-1990s led to significant positive changes in the natural history of HIV infection. With ongoing improvements in treatment and overall clinical management of HIV and its most common comorbidities, people with HIV in countries with accessible medical care now have life expectancies comparable to those of the general population. While the clinical management of HIV has led to longer life and improved overall health, stigma and discrimination remain pervasive issues in the lives of people with HIV in Canada and worldwide.

Combination antiretroviral therapy remains the greatest success in the field of HIV, and advances in HIV care have continued over the past 20 years. Today, HIV treatment includes seven classes of medications, more than 30 total medications and countless combinations to produce dozens of regimens that can meet the unique needs of a person with HIV (*Cohen et al., 2016*). Regimens typically include three medications from two different classes of antiretrovirals. Treatment has progressed to now include 10 single-tablet regimens that allow for one pill, once-a-day HIV treatment for many people with HIV, likely to be more in the future. The desired treatment outcome of combination antiretroviral therapy is full plasma viral suppression of HIV in the infected person. With an effective regimen, greater than 90% medication adherence and adequate support, this outcome is highly achievable.

The ability to achieve and sustain full viral suppression marks another paramount advancement for people with HIV because of the impact it has on transmission. By 2019, over 850 scientific agencies had endorsed the position that 'people living with HIV on antiretroviral therapy with an undetectable viral load in their blood have a negligible risk of sexual

transmission of HIV' ('CONSENSUS STATEMENT | United States | Prevention Access Campaign,' n.d.). The statement continues with an explanation of negligible to mean 'so small or unimportant as to be not worth considering'. This follows the 2017 statement from the Centers for Disease Control (CDC): 'when antiretroviral therapy results in viral suppression, defined as less than 200 copies/ml or undetectable levels, it prevents sexual HIV transmission' (<https://www.cdc.gov/hiv/pdf/risk/art/cdc-hiv-art-viral-suppression.pdf>).

Across the two largest studies with heterosexual couples (HPTN 052 [*Cohen et al., 2016*] and PARTNER [*Rodger et al., 2016*]) (TABLE 1), which included thousands of couples and many thousand acts of sex, mostly without condom use and none having received pre-exposure prophylaxis, no linked HIV transmissions to an HIV-negative partner were observed when the HIV-positive person was virally suppressed. This fact was further confirmed in two studies with large samples of same-sex male couples (*Rodger et al., 2016*). This means that people who take antiretroviral therapy daily as prescribed, and achieve and maintain an undetectable viral load, have effectively no risk of sexually transmitting the virus to an HIV-negative partner.

This messaging has had significant impact on the lives of people with HIV and their partners, and has particular implications for conception. Canada's Department of Justice has issued a directive that federal prosecutors should not prosecute for HIV non-disclosure when the person living with HIV (*Canada, 2018*) has maintained a suppressed viral load (i.e. under 200 copies of the virus per millilitre of blood) because there is no realistic possibility of transmission (<https://ppsc-sppc.gc.ca/eng/pub/fpsd-sfpg/fps-sfp/tpd/p5/ch12.html>). This is based on the aforementioned studies and an additional meta-analysis commissioned by Public Health Canada (*LeMessurier et al., 2018*).

#### **Provision of ART when there is an undetectable HIV viral load**

When an ejaculate is deemed non-infectious with intercourse, it is also non-infectious at all points of processing in the ART laboratory. This means that no additional precautions such as physical separation of patients or samples are required beyond universal precautions and standard IVF laboratory practice.

Concerns have been voiced regarding the applicability of these landmark HIV studies to the pragmatic world, as they only reported on patients in care, presumably adherent to treatment, with persistently undetectable viral loads. The rate of non-adherence varies among populations, and the possibility of non-adherence in the infertility population is real. The supplemental analysis from the PARTNER (*Rodger et al., 2019*) study provides reassurance as to the safety of this approach. It notes that if the periods of follow-up time (defined between two consecutive HIV tests) in which the HIV RNA was suppressed at the beginning of the period but during which the HIV RNA became elevated were included, the number of within-couple transmissions was still zero.

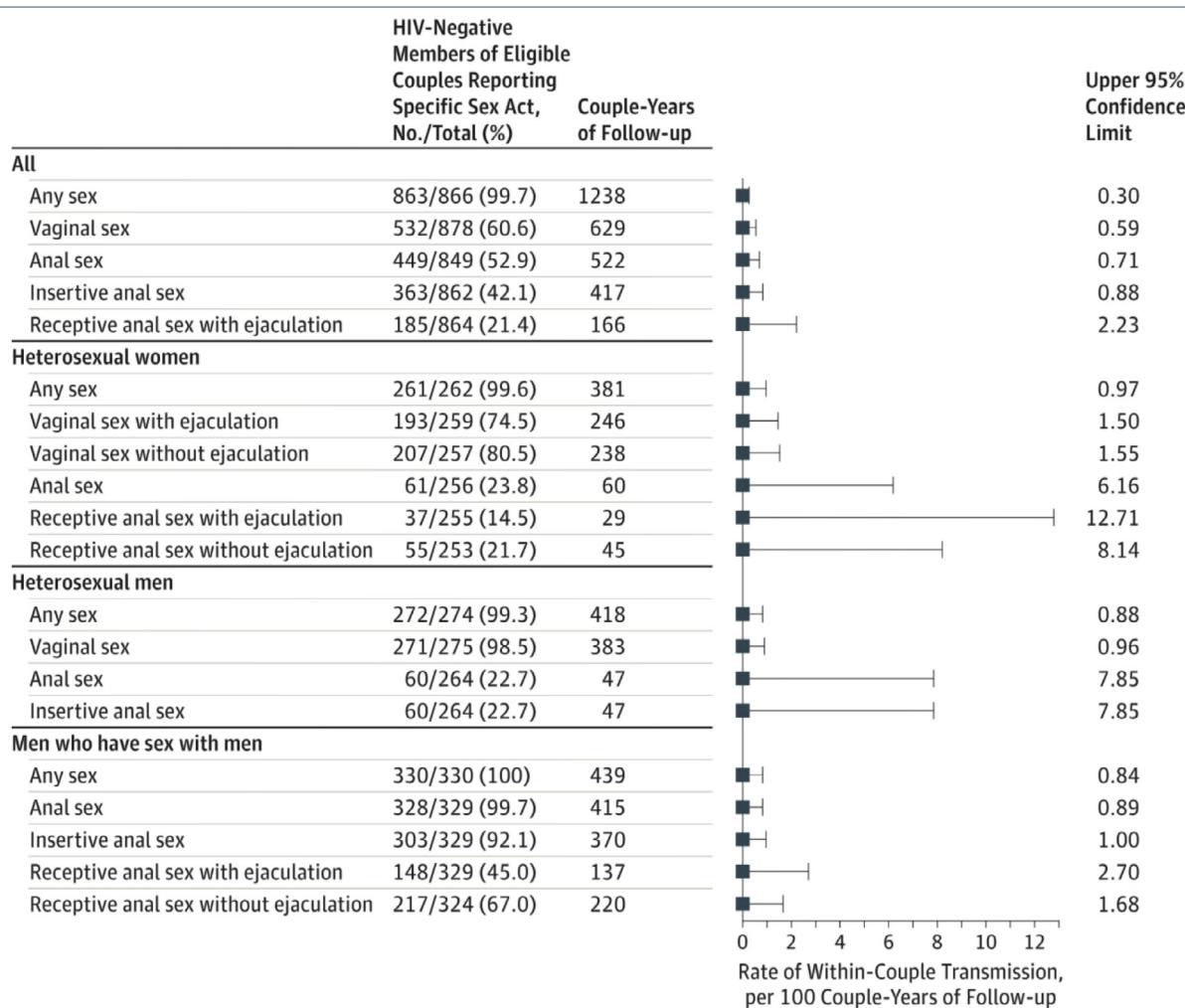
#### **Sensitivity Analysis**

Sensitivity analysis was conducted including the period of follow-up time in which the HIV-RNA was suppressed at the beginning of the period, but during which it became elevated (eTable 3). If we include the periods of follow-up time (defined between two © 2016 American Medical Association. All rights reserved. consecutive HIV tests), in which the HIV-RNA was suppressed at the beginning of the period but during which the HIV-RNA became elevated, the number of within-couple transmissions is still zero, therefore the estimated rate is zero. The estimates / confidence intervals included in the manuscript and the alternative described above are shown in the table below.

This is consistent with previous data showing that the risk per individual sexual act is in the realm of 0.004–0.008% (*LeMessurier et al., 2018*). The implication of the CDC recommendation is that all providers of ART should provide all services to people with HIV who have a sustained undetectable viral load.

#### **Recommendations:**

- 1 A person living with HIV should be under the regular care of an HIV-care provider prior to beginning fertility treatment.
- 2 A person living with HIV should be on combination antiretroviral therapy for a minimum of 3 months, preferably 6 months, and have two suppressed viral load results (i.e. <200 copies/ml or 'target not detected') at least 1 month apart, and then every 6 months

**TABLE 1 PARTNER STUDY CHARACTERISTICS AND RESULTS**

(or more frequently if questions of adherence arise or at the discretion of their HIV-care provider) prior to attempting conception.

3 ART laboratories should not offer sperm washing and IUI solely to reduce the risk of horizontal transmission if the spermatozoa come from a person living with HIV who has been on combination antiretroviral therapy for a minimum of 3 months (preferably 6 months) and has had two suppressed viral load results (i.e. <200 copies/ml or 'target not detected') at least 1 month apart.

#### Provision of ART when there is a detectable HIV viral load

There is expert consensus that pregnancy should be deferred until infected partners are adequately treated (Loutfy et al., 2018). However, there may be circumstances where this is not possible,

and ultimately it is the patient's decision. They should be supported in making informed, autonomous decisions about their health and be offered appropriate counselling to do so in the context of ART. When viral load in the female partner is not adequately suppressed, the SOGC guidelines for reducing perinatal transmission should be followed and IUI offered (Money et al., 2014).

#### Managing the risk of horizontal transmission with HIV

There are several studies addressing the situation in which the male partner is living with HIV with a detectable serum viral load and IUI has been performed. For the purposes of this guideline, reports not written in English and those that did not separate IVF/intracytoplasmic sperm injection from IUI were excluded. However, there are a number of meta-analyses including such

papers that demonstrate the efficacy of sperm washing as a risk-reduction method when there is a detectable HIV viral load.

A 2011 meta-analysis found no reports of transmission across 3900 cycles of IUI in 1184 HIV-serodiscordant couples (Vitorino et al., 2011). In a 2014 meta-analysis based on 8212 seropositive male and seronegative female IUI cycles and 1254 seropositive male and seronegative female IVF cycles (Barnes et al., 2014), the authors found no reports of transmission. The 95% confidence interval (CI) would suggest an upper limit rate of 4.5 transmissions per 10,000. A larger meta-analysis published in 2016 reported on 11,237 cycles (Zafer et al., 2016). Of the couples treated, 27.7% of the males had not achieved viral suppression at the time of semen washing. There were no HIV seroconversions among this subset

**TABLE 2 SPERM WASH AND IUI FOR REDUCTION OF HORIZONTAL TRANSMISSION OF HIV**

Certainty assessment					Summary of findings	Certainty	Importance			
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations				
HIV detection after standard sperm washing										
4	Observational studies	Serious <sup>a</sup>	Not serious	Not serious	Not serious	None	Serodiscordant couples (male HIV infected). 107 sperm samples washed; HIV detected after washing in 5.6% of samples, which were not used. No seroconversions in 63 patients with 101 cycles of IUI (Marina et al., 1998) 741 serodiscordant couples (male HIV infected). 2871 sperm washing procedures. 581 IUI cycles After sperm washing, virus detected in 4% of samples, which were not used. No seroconversions in 581 patients (Savasi et al., 2007) 1036 serodiscordant couples (male HIV infected), 3390 cycles of assisted reproduction (IUI, IVF, ICSI and frozen embryo transfer) with sperm washing; no seroconversions in 967 women (with 3272 cycles) tested 6 months after procedure (loss to follow up 71%) (Bujan et al., 2007), serodiscordant couples (male HIV infected) undergoing 294 IUI cycles with sperm washing; no seroconversions in all 84 patients (Bujan et al., 2004) Retrospective analysis of 631 serodiscordant couples with 2113 sperm washing IUI cycles with no seroconversions (loss to follow-up testing 90 days after insemination for 26%) (Semprini et al., 2013)	⊕⊕⊕○	Strong	High

<sup>a</sup> Risk of bias is serious due to bias inherent to observational studies.

HIV, human immunodeficiency virus; ICSI, intracytoplasmic sperm injection; IUI, intrauterine insemination.

of 1023 couples. The per-cycle risk of HIV seroconversion was zero. The 95% CI with this larger sample reduces the risk to a maximum of 0.0006% per cycle. The sperm preparation method varied among the studies, as such none is clearly proven to be superior. Today the standard protocol is gradient and swim-up as described by Semprini and the European CREAthe network (Semprini et al., 1992). A double-tube method that allows the sperm pellet to be retrieved after centrifugation, without coming into contact with the gradient layers and seminal plasma, has also been developed but is not widely used (Fourie et al., 2015).

The aforementioned meta-analyses referenced papers that are not available in English. The four English language observational studies can be found in TABLE 2. While observational studies have weaknesses, the large sample size, the long period of use of the techniques and the multiple sites reporting outcomes leads to a strong recommendation

for the use of gradient and swim-up techniques and IUI in serodiscordant couples.

#### Recommendations:

- 4 ART providers should offer IUI to reduce the risk of female-to-male transmission if the female partner cannot achieve an undetectable HIV viral load.
- 5 ART laboratories should process ejaculates from men unable to achieve an undetectable HIV viral load using gradient and swim-up techniques to reduce the risk of horizontal transmission.

#### Managing the risk of HIV cross-contamination when processing spermatozoa

Double-washing has been well proven and universally used for HIV-positive specimens; thus, there are no clinical studies addressing the question of whether single-washing renders specimens non-infectious. There is potential for cross-contamination in the intermediary steps of sperm washing, with various semen fractions and with spent media. Small studies have found one sample wash positive after the initial process (Fiore et al., 2005; Savasi et al., 2010). By spiking samples with HIV, Fiori and colleagues demonstrated that, depending on the viral load, spent media may harbour HIV. Accurate estimates of the risk of cross-contamination based only on the risk of virus in spent media cannot be inferred but do provide a start. When a sample from a person known to have a detectable viral load is processed, the resulting sample is unlikely to harbour virus and the absolute load is

**TABLE 3 HIV IN SPENT MEDIA**

Certainty assessment						Summary of findings	Certainty	Importance	
No. of studies	Study design	Risk of bias <sup>a</sup>	Inconsistency	Indirectness	Imprecision	Other considerations			
Viral detection across different semen fractions									
3	Observational studies	Serious <sup>a</sup>	Not serious	Serious	Serious	None	Semen samples from HIV-infected patients ( $n = 12$ ); after density gradient centrifugation, one sample where virus was detected; after swim-up, all samples tested negative for viral detection (Hanabusa et al., 2000) Semen samples ( $n = 16$ ) from HCV/HIV-infected patients; detected virus in raw semen, seminal plasma and non-sperm cells, but not after density centrifugation or swim-up (Savasi et al., 2010) Semen samples ( $n = 62$ ) with HIV/HBV/HCV; washed with gradient centrifugation (no swim-up) and tested negative for viral detection (Molina et al., 2014)	⊕OOO Very low	Critical

<sup>a</sup> Risk of bias is inherent due to observational study design.

<sup>b</sup>Small sample: Hanabusa and colleagues (Hanabusa et al., 2000) had  $n = 12$  semen samples from HIV infected patients, and Savasi and co-workers (Savasi et al., 2010) had  $n = 16$  semen samples from HIV/HCV-infected patients.

HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus.

very low. This can cause infection only if it also comes into direct contact with a receptive host tissue; when samples are processed separately, this cannot happen. The studies in TABLE 3 provide only indirect evidence about the risk of cross-contamination but are nonetheless reassuring.

#### Recommendations:

- 6 When recommendations 1 and 2 are met, and ART is being used for a fertility indication, ART laboratories should not provide any additional sperm processing solely to reduce the risk of cross-contamination.
- 7 When a person is unable to reach an undetectable HIV viral load, and ART is required, ART laboratories should process ejaculates as the only specimen in the hood or centrifuge in order to reduce the risk of cross-contamination, and should follow cleaning and disinfection procedures between samples as set by provincial regulations.

#### Managing the risk of HIV cross-contamination when processing oocytes

Oocyte retrieval requires puncture of tissue with ensuing microscopic or macroscopic bleeding; thus, there is potential for contamination with the procedure. However, Health Canada clearly states that standard equipment and room cleansing is acceptable for reducing cross-contamination risk. This is likewise true in the laboratory, where

follicular fluid and blood are washed from the oocyte. Standard preparation of the egg for insemination or vitrification has been demonstrated to effectively remove viral particles from the follicular fluid (Cobo et al., 2012). The rate of HIV DNA retrieval decreases from 89% to 25% by day 1 after oocyte retrieval, and DNA becomes undetectable after washing the embryos and refreshing the media, irrespective of the original bloody status of the follicular fluid.

There is no evidence to suggest that the oocyte of an HIV-positive woman can be infected by the virus, as CD4, and other HIV receptors, have never been demonstrated in mature oocytes or follicular cells (Baccetti et al., 1999). The oocyte is known to be a poor vector of pathogen agents because, in addition to the barrier effect of the zona pellucida, it is an isolated cell with no blood supply.

#### Recommendation:

- 8 When a person is unable to reach an undetectable HIV viral load, and ART is required, ART laboratories should process oocytes as the only specimen in the hood in order to reduce the risk of cross-contamination, and should follow cleaning and disinfection procedures between samples as set by provincial regulations.

#### Managing the risk of HIV cross-contamination with embryo culture

The risk of cross-contamination in the incubator is based on the potential

infectivity of the gamete, the likelihood of its media coming into direct contact with another gamete, and the potential of the recipient gamete to become infected. As has been shown, washing for HIV-infected spermatozoa renders them non-infectious when a double process is used. Although there are not have the same large-scale observation studies for oocytes, the same appears to hold true (Cobo et al., 2012). Thus, embryos created from individuals and couples affected by HIV are themselves non-infectious. However, even in the unlikely event that the virus was not completely removed, the risk of cross-contamination in the incubator (i.e. coming into direct contact with another gamete) is also determined by the type of incubator (single chamber box versus individual chamber) and the number of patients per chamber. The use of one chamber per patient eliminates any risk of cross-contamination while in the incubator. However, the common practice of culturing under oil droplet also serves to minimize the chance of microbial contamination.

Finally, after removal from the incubator prior to fresh embryo transfer, trophectoderm biopsy or vitrification, the usual practice is to wash the embryo and replace the media. This final step would again provide an opportunity remove any potential contamination from the media.

In summary, given the many opportunities to wash gametes of virus

before embryo transfer, the likelihood of contamination, even with the potential of spillage within the incubator, is negligible.

#### **Recommendation:**

- 9 ART laboratories may culture embryos from a person with a detectable HIV viral load using standard processes in individual chambered incubators or box incubators.

### **PROVISION OF ART WHEN THERE IS HEPATITIS C INFECTION**

#### **Background**

Approximately 0.67% of Canadians are infected with HCV. This varies from 3% in Indigenous populations to 0.8% in the cohort born from 1950 to 1975 and is extremely low in younger age groups (*Shah et al., 2018*). It is the most common newly diagnosed BBVI today in Canada, although there are more people living with chronic HBV infection (*Canada, 2014*).

The current understanding of diagnosing and managing HCV has altered dramatically since its discovery in 1989 (*Choo et al., 1989*). HCV is a blood-borne infectious disease that is caused by an RNA virus and primarily affects the liver. Often, patients are asymptomatic. If left untreated, 5–20% of individuals infected with HCV spontaneously clear the virus, and these individuals are non-infectious. However, for the remaining 80%, HCV becomes chronic and may lead to consequences such as cirrhosis of the liver, liver cancer and, after decades, even liver failure.

The first treatment for HCV became available in 1991, with a cure rate of less than 10% (*Poynard, 2004*). However, the treatment landscape has made tremendous advances with the use of direct-acting antivirals. HCV treatment is guided by factors such as viral genotype, prior antiviral use, liver cirrhosis, HIV or HBV co-infection and pregnancy. Current treatment with glecaprevir/pibrentasvir for as little as 8 weeks or with sofosbuvir/velpatasvir for 12 weeks can confer greater than 90% cure rates (*Grover and Erlich, 2018*). A person is considered cured of HCV, and un-infectious, if there is no detectable virus ('target not detected') in a serum sample 3 months after completing HCV treatment. HCV antibody positive but RNA-negative individuals do not need any specific

intervention to reduce transmission as they are non-infectious.

#### **Recommendations:**

- 10 A person with quantifiable HCV (detectable HCV RNA) should be treated for HCV before conceiving.
- 11 If/once the HCV RNA of a gamete provider is unquantifiable, ART laboratories should not use any additional spermatozoa, oocyte or embryo processing solely to reduce the risk of horizontal transmission.
- 12 If a gamete provider is HCV RNA unquantifiable, regardless of the HCV antigen or antibody status, ART providers should not offer specific processes solely to reduce the risk of cross-contamination in the laboratory.

#### **Managing the risk of horizontal transmission of HCV**

It is recommended that people be cured of HCV before attempting conception. However, there may be circumstances when this is not possible, such as lack of drug access or advanced reproductive age. The presence of HCV in the semen of infected men is well documented. However, the presence of virus is not sufficient to cause infection. HCV is not a DNA virus (unlike HBV) and has no reverse transcriptase activity (unlike HIV); it therefore cannot integrate into spermatozoa or embryo DNA, which significantly reduces concern about horizontal transmission (*Dodge and Terrault, 2014*). Furthermore, several studies of thousands of people-years have documented negligible transmission rates in the absence of co-infection with HIV (*Bresters et al., 1993; Terrault et al., 2013; Tohme and Holmberg, 2010*). Thus, the recommendation is that a condom is not necessary for penile-vaginal intercourse in HCV serodiscordant couples, with the understanding that activities that have the potential for blood-to-blood contact do allow for transmission.

Despite laboratory evidence of the presence of virus, when unprotected intercourse is deemed safe, no additional measures are needed in the ART laboratory to prevent horizontal transmission.

#### **Recommendation:**

- 13 If either partner has active HCV but no other BBVI, ART providers should not offer IUI solely to reduce the risk of horizontal transmission.

#### **Managing the risk of HCV cross-contamination when processing spermatozoa**

In men infected with HCV, HCV RNA has been detected in different semen fractions (*Garrido et al., 2005*) but sperm washing has been shown to be effective at reducing the virus, as outlined for HIV above. In contrast to the HIV literature, preparation using only Percoll has been shown to eliminate HCV RNA (*Halfon et al., 2006*). The likelihood of cross-contamination, given the virus characteristics, is very small with routine processes.

#### **Recommendation:**

- 14 If a sperm provider has quantifiable HCV and requires ART for fertility purposes, ART laboratories may process the ejaculate as the only specimen in the hood or centrifuge in order to reduce the risk of cross-contamination, and follow cleaning and disinfection procedures between samples as set by provincial regulations.

#### **Managing the risk of HCV cross-contamination with oocyte processing**

Potential contamination with oocyte processing is likely as a result of blood in the follicular fluid, which is removed during the process identifying the oocyte and preparing for insemination (*Cobo et al., 2012; Devaux, 2003; Levy, 2000*).

#### **Recommendation:**

- 15 If an egg provider has quantifiable HCV and requires ART for fertility purposes, ART laboratories should process oocytes as the only specimen in the hood in order to reduce the risk of cross-contamination, and should follow cleaning and disinfection procedures between samples as set by provincial regulations.

#### **Managing the risk of HCV cross-contamination with embryo culture**

Despite the potential for HCV to be found in the follicular fluid, the likelihood of persistence in embryo culture is low. As for all BBVI, the risk of cross-contamination in the incubator is based on the potential infectivity of the gamete, the likelihood of its media coming in direct contact with another gamete, and the potential of the recipient gamete to become infected. As has been shown, washing HCV-infected spermatozoa or eggs renders

them non-infectious, although there are few data for eggs (Cobo *et al.*, 2012). Thus, embryos created from individuals and couples with HCV are likely to be non-infectious. However, even in the unlikely event that virus was not completely removed, the risk of cross-contamination in the incubator (i.e. coming into direct contact with another gamete) is also determined by the type of incubator (box versus individual chamber) and the number of patients per chamber. The use of one chamber per patient eliminates any risk of cross-contamination while in the incubator. Another level of protection against cross-contamination during embryo incubation is attained by the culturing in media drops under oil.

Finally, after removal from the incubator prior to fresh embryo transfer, trophectoderm biopsy or vitrification, the usual practice is to wash the embryo and replace the media. This final step again would again provide opportunity remove any potential contamination from the media.

#### **Recommendation:**

16 ART laboratories may culture embryos from a person with a detected HCV RNA using standard processes in individual chambered incubators or box incubators.

#### **PROVISION OF ART WHEN THERE IS HEPATITIS B INFECTION**

##### **Background**

The virus for hepatitis B, and the subsequent vaccine, was discovered by Nobel laureate Baruch Blumberg in 1965. More than 240 million individuals worldwide are chronically infected

with HBV, which can lead to hepatitis, cirrhosis, fulminant liver failure and hepatic cancer. In Canada, cases of chronic HBV infection are falling, with an overall rate of 11/100,000 but one as high as 28/100,000 in men aged 20–29 years (Aparna *et al.*, n.d.). HBV is most common in central and east Asia, sub-Saharan Africa and the Pacific (5–8% adults), as well as other developing countries and low-income communities without the resources or financial support for vaccination programmes, screening tools or treatment programmes. HBV continues to be an issue in Canada among immigrants from countries where HBV is endemic (Canada, 2019). Interpretation of hepatitis B testing can be found in TABLE 4.

Successful immunization provides effective protection against HBV infection. However, 5–15% of individuals do not respond to the vaccination and revaccination is recommended. If vaccination is still unsuccessful, these individuals should be counselled on prevention of acquiring and transmitting HBV.

Antiviral therapy is the primary form of treatment for those with chronic infection. The goal of HBV treatment is to prevent liver cirrhosis and cancer. Specific treatment is determined by aspartate aminotransferase and alanine aminotransferase concentrations, HBV DNA level and degree of fibrosis. Other considerations include patient age (adult or child), main genotype and other co-infections. Treatment of HBV may be lifelong and is best managed by an HBV expert.

HBV is orders of magnitude more infectious than HIV. HBV is transmitted

by percutaneous or mucosal exposure to infected blood or other body fluid. Transmission has been observed with numerous forms of human contact such as perinatal/mother to child, household (non-sexual), sexual, needle sharing and occupational/health-care-related. The CDC reported 65 outbreaks (two or more cases) of viral hepatitis related to healthcare during 2008–2018; of these, 60 (92%) occurred in non-hospital settings. Twenty-five outbreaks were of hepatitis B, which resulted in 183 outbreak-associated cases. The majority occurred at long-term facilities with breaches in glucose monitoring process ('Outbreaks Related to Healthcare 2008–2015 | Statistics & Surveillance | Division of Viral Hepatitis | CDC,' 2019).

HBV is most commonly transmitted sexually and perinatally. An infection rate of 20–40% is seen in the regular heterosexual partners of HBV-infected patients (Brook, 2002). HBV genotypes A and C are most common in individuals who acquire HBV sexually (Aparna *et al.*, n.d.). The risk of horizontal transmission of HBV is completely eliminated when the partner is immune. However, the person carrying the pregnancy may not have been vaccinated or show immunity after vaccination (Bakker *et al.*, 2016). Thus, the question of the infectivity of spermatozoa remains germane. HBV DNA or RNA can be extracted from spermatozoa and only occasionally from resulting embryos in humans (Ali *et al.*, 2006).

HBV DNA can integrate into the germline. However, the presence of DNA in the germline does not necessarily result in vertical transmission (Hadchouel *et al.*, 1985; Nie *et al.*, 2011). Jin and colleagues (Jin *et al.*, 2016) reported

**TABLE 4 INTERPRETATION OF HEPATITIS B TESTING**

	Hepatitis B surface antigen (HBsAg)	Total hepatitis B core antibody (anti-HBc)	IgM antibody to hepatitis B core antigen (anti-HBc IgM)	Hepatitis B surface antibody (anti-HBs)	Hepatitis B e antigen (HBeAg)	Antibody to hepatitis B e antigen (anti-HBe)
Acute or chronic infection	+	+	+	–		
Carrier, infectious	+	+	–	–	+	–
Carrier, low infectivity	+	+	–	–	–	+
Past infection, immune	--	+		+		
Remote or false positive	–	+	–	–		
Vaccinated, immune	–	–	–	+		
No infection, susceptible	–	–	–	–		
Rare	+	+		+	+/-	+/-

IgM, immunoglobulin M.

on excess embryos or unfertilized eggs and the babies resulting from sibling embryos, these babies being tested for HBV. Twelve babies were born to HBsAg-positive couples, and were all HBsAg-negative, even when unfertilized oocytes and non-viable embryos from these same couples were HBV positive. A study of HBV-negative pregnant women with a paternal HBV carrier (i.e. sperm provider) undergoing amniocentesis did not demonstrate any HBV infection in the livers of eight fetuses that were aborted (for other reasons) (Cai and Zhu, 2013). There was no evidence of paternal hepatitis antigen in over 100 samples, suggesting that paternal transmission is not a concern. This suggests that either the spermatozoa were not infective or the lack of liver development until 12 weeks of gestational age allowed for maternal clearance before any infection, or that infection was incompatible with ongoing pregnancy (Ye et al., 2013). The very small sample size precludes definitive conclusions about lack of sperm infectivity so this question remains.

### **Managing the risk of horizontal transmission of HBV**

#### **Recommendations:**

- 17 If either reproductive partner is HBV surface antigen positive:
  - (a) The infected partner should be referred to an HBV-care provider.
  - (b) The other partner must be tested for HBV surface antigen, surface antibody and core antibody, and be immunized if not immune.
  - (c) The couple should be counselled to use condoms until the HBV-susceptible partner is immune.
- 18 When the gestating partner is HBV immune and the ejaculate is from a person infectious for HBV, ART providers should not offer IUI solely to reduce the risk of horizontal transmission.
- 19 When the gestating partner is HBV immune and the ejaculate is from a person infectious for HBV, and IUI is needed for fertility purposes, ART laboratories should prepare the spermatozoa in the usual fashion with a single wash.

If the sperm provider is HBV surface antigen positive and the gestating partner is non-immune (after vaccination), there are no data to guide the choice of single or double sperm washing to reduce

horizontal transmission. HBV DNA or RNA can be extracted from spermatozoa and sometimes from resulting embryos in humans (Cai and Zhu, 2013). However, HBV is present in a much higher amount in the seminal fluid. Thus, sperm washing is still appropriate to reduce horizontal transmission. Alternatively, the person providing the spermatozoa can be considered for virus-reducing therapy.

- 20 When the gestating partner cannot achieve HBV immunity and the ejaculate is from a person infectious for HBV, ART providers may use gradient and swim-up for IUI to reduce the risk of horizontal transmission.
- 21 When the gestating partner is infectious for HBV, and the sperm provider is non-immune, IUI may be used to reduce the risk of horizontal transmission.

### **Managing the risk of cross-contamination with HBV**

We are not aware of any studies in which environmental surfaces have been clearly associated with the actual spread of HBV and HCV. However, it is well established that, without proper cleaning, HBV can survive outside the body for at least 7 days and is at least theoretically capable of causing infection (Sattar et al., 2001).

#### **Recommendations:**

- 22 ART laboratories should process gametes from a person infectious for HBV separately, and follow cleaning and disinfection procedures between samples as set by provincial regulations.
- 23 ART laboratories may culture embryos from a person infectious for HBV using standard processes in individual chambered incubators or box incubators.

### **Managing risk of cross-contamination with cryopreservation**

Cryostorage has intrinsic risks, not only of microbiological contamination but also risks including, but not limited to, equipment failure, thawing and leakage of genetic material. The infectious risk is not limited to BBVI (Bielanski et al., 2003; Joaquim et al., 2017). As with the risk of embryo culture, opportunities for cross-contamination exist if the sample is infectious, if it can come into direct contact with the recipient gamete, and if the recipient gamete cannot be cleared of the BBVI before transfer.

However, there are fundamental differences between culture and storage. Embryo culture is for a duration of days. Cryopreservation may span years, so the cumulative risk of any problem is magnitudes bigger. Best practices mandate provisions for known and currently unknown risks. The addition of BBVI samples should not change risk management. As previously discussed with respect to embryo culture risk, it is unlikely that gametes that have been washed and prepared in the usual way for cryopreservation are infectious. Again, the preparation after thaw, and before transfer, further reduces the risk.

The storage of gametes or embryos in straws is akin to separate chambers of incubators. This is referenced in US regulatory standards. Of note, Pomery stated in 2010:

According to the FDA, storage is considered to be improper if unique specimens occupy the same location in such a way that provides the opportunity to mix, cross-contaminate, or be inadvertently distributed. It cannot be emphasized enough that it is permissible to physically locate specimens in the same storage tank provided that the aforementioned safeguards are assured. (Pomery et al., 2010)

Health Canada has recently clarified their position, which is likewise that separation of specimens of unknown status is administrative, not physical, and does not require separate equipment. This means that samples of known positive BBVI status can be stored along with other samples.

A sample from individuals with a BBVI poses a theoretical risk with cryopreservation. Most micro-organisms can survive storage at liquid nitrogen temperatures, and the cryoprotectants used in embryo and oocyte cryopreservation may also protect viruses. However, infection from liquid nitrogen in the ART setting has not been demonstrated. Liquid nitrogen tested from stored oocytes and embryos ( $n = 27$ ) of HIV-, HBV- and HCV-infected patients did not demonstrate any virus in any sample (Cobo et al., 2012). This is likely to reflect the aforementioned ability to remove virus during the washing process. TABLE 5 summarizes the available studies.

The risk of cross-contamination from cryopreservation is also dependent on

**TABLE 5 RISK OF CROSS-CONTAMINATION WITH CRYOPRESERVATION**

Certainty assessment					Summary of findings		Certainty	Importance
Nº. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations		
Risk of contamination into liquid nitrogen								
1	Observational studies	Serious <sup>a</sup>	Not serious	Not serious	Serious <sup>b</sup>	None	Tested liquid nitrogen ( $n = 27$ ) from oocyte ( $n = 14$ ) and embryo vitrification ( $n = 10$ ) and liquid nitrogen storage tank samples ( $n = 3$ ) from HIV-, HBV- and HCV-infected patients; all samples tested negative for viral detection (Cobo <i>et al.</i> , 2012).	⊕OOO Very low
Cryopreservation straws for storage of HIV-infected sperm samples								
1	Observational studies	Serious <sup>a</sup>	Not serious	Serious <sup>c</sup>	Not serious	None	Compared rinse media from IR ( $n = 24$ ), PVC ( $n = 24$ ) and PETG straws ( $n = 24$ ) filled with HIV-1 supernatant and kept in liquid nitrogen for 7 days; no viral detection in rinse media from IR straws (Letur-Konirsch <i>et al.</i> , 2003)	⊕OOO Very low
Cryopreservation straws for storage of HCV-infected sperm samples								
1	Observational studies	Serious <sup>a</sup>	Not serious	Serious <sup>d</sup>	Not serious	None	Rinse media from 102 IR straws filled with uninfected seminal plasma spiked with HCV-infected blood plasma compare at three concentrations kept in liquid nitrogen for 7 days; no viral detection in rinse media from IR straws (Maertens, 2004)	⊕OOO Very low
Risk of cross-contamination with shared storage of hepatitis B-infected blood products								
2	Observational studies	Serious <sup>a</sup>	Not serious	Very serious <sup>e</sup>	Very serious <sup>f</sup>	None	Report cross-contamination from leaked bag of HBV-infected bone marrow sample to six uninfected samples (Tedder <i>et al.</i> , 1995) Leaking HBV blood product sample in combined storage with uninfected samples; testing of 115 uninfected samples showed negative for viral detection (Husebekk <i>et al.</i> , 2004)	⊕OOO Very low

<sup>a</sup> Risk of bias is inherent due to observational study design.

<sup>b</sup> Small sample:  $n = 27$  liquid nitrogen samples from vitrification and storage tanks tested.

<sup>c</sup> Used HIV-infected supernatant but not sperm samples from infected patients (Letur-Konirsch *et al.*, 2003).

<sup>d</sup> Used sperm samples from uninfected patients spiked with blood plasma from HCV-infected patients but not sperm samples from infected patients (Maertens *et al.*, 2004).

<sup>e</sup> Tedder and colleagues (Tedder *et al.*, 1995) report cross-contamination to uninfected bone marrow samples from HBV-infected samples, and Husebekk and co-workers (Husebekk *et al.*, 2004) report no contamination with shared storage of blood products.

<sup>f</sup> Tedder and colleagues (Tedder *et al.*, 1995) provide a case report of a single event of contamination from one HBV-infected sample to five other samples; Husebekk and co-workers (Husebekk *et al.*, 2004) report no contamination from one leaked bag to 55 other samples.

HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus. PCR polymerase chain reaction an in vitro technique for rapidly synthesizing large quantities of a given DNA segment that involves separating the DNA into its two complementary strands, using DNA polymerase to synthesize two-stranded DNA from each single strand, and repeating the process –abbreviation PCR; PETG Polyvinyl chloride (PVC), polyethylene terephthalate glycol IR high-security ionomeric resin

the type of storage method – liquid or vapour phase – and the type of straw – open or closed. Vapour-phase storage eliminates the vector for contamination. While it may be superior from an infection control perspective, it may present greater risk of compromise or loss of cryopreserved specimens (Vajta *et al.*, 2015). Studies using washing techniques after thaw have demonstrated complete removal of bacteria and fungi, and this would be likely to be true of BBVI (Parmegiani *et al.*, 2012).

In a closed liquid device, the nitrogen of the common container is never in direct contact with biological material frozen

on the inside, so cross-contamination cannot occur unless there are several breaks in the system. If this were the case, the stored biological material would be at material risk. After loading in a closed system, the plastic straw can be heat-sealed on both ends, and consequently the solution containing the embryo and the liquid nitrogen are hermetically isolated during cooling and storage system. Closed systems reduce the potential danger of contamination through liquid nitrogen, but open systems provide direct contact for the required cooling and warming rates for vitrification. Contamination in animal models under experimental conditions

has been shown (Bielanski *et al.*, 2000). However, a review by the same authors acknowledges the differences between these studies and human practice (Bielanski and Vajta, 2009). The risk of direct contact may be ameliorated by the effects of the cryoprotectants against contamination.

Assessing risk of cross-contamination can only be done indirectly by measuring infectious material in liquid nitrogen after routine processing. There are no studies or reports of tank failure that have resulted in contamination. Sadly, there are reports of tank failure that have resulted in embryo demise.

**TABLE 6** BASELINE RATE OF SEROPOSITIVE INDIVIDUALS IN AN INFERTILITY POPULATION

Study	Hart et al. (2001)	Abusheikha et al. (1999)	Hughes et al. (2011)	Pepas et al. (2011)	Wingfield and Cottell (2010)	Gold et al. (2019)
n	815	4960	12,700	3910	13,717	1945
HIV (%)	0.13	0.06	0.007	0.6	0	0
HCV (%)	0.5	0.5	0.33	0.4	0.001	0.008
HBV antigen (%)	1.3	0.5	0.28	1.7	0.001	0.01
Conversion rate (all)	N/A	0	0	0	0	0

HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus.

#### Recommendation:

24 ART laboratories storing cryopreserved gametes or embryos with a potential BBVI transmission risk (i.e. untested or with a detectable serum viral load), can use a closed or open system with vapour-phase storage or a closed system with liquid phase to mitigate potential equipment failure, but do not need to use separate tanks.

#### Managing cross-contamination with testing frequency

The current practice of many Canadian fertility clinics is mandatory annual testing for BBVI in all users of ART (IVF and IUI). This does not reflect evidence-based guidelines that base the frequency of testing on risk factors, but instead reflects a misplaced fear of cross-contamination between specimens in the ART setting, and an attempt at risk management on the part of the ART providers. An unintended consequence of this approach has been to perpetuate the stigma associated with BBVI, especially HIV, and limit the access to care of affected individuals.

Canadian Guidelines (HIV Screening and Testing Guide Canada.ca) recommend preconception BBVI testing of all

individuals and repeat testing of low-risk individuals only if risk factors change, or at the request of the patient. Annual testing is unwarranted for the majority of ART patients, despite it being the practice of virtually all ART units, which believe it will reduce risk of cross-contamination.

Maintaining the mandatory BBVI testing in the ART setting but lengthening the time between testing possibly increases the number of undiagnosed cases that are being processed in an ART laboratory. Eliminating repeat testing would increase the risk of undiagnosed cases over the whole period of fertility care, which may be years.

Data are not available on new cases of BBVI that have been diagnosed during fertility treatment in Canada. A UK study in 2001 demonstrated a baseline rate of HIV and HBV of 0.8% and 1.4%, respectively, in the infertile population, similar to the prenatal testing rates of 0.13% and 1.7 (Hart et al., 2001). A larger study demonstrated HBV and HIV baseline risks of 0.28% and 0.007% with no additional cases diagnosed over the course of treatment of 12,000 individuals (Abusheikha et al., 1999). Two smaller studies could not demonstrate any new

cases among over 600 patients who had repeat testing within 3 years. These are summarized in TABLE 6. In Canada, the prevalence of HIV is 173/100,000 population (Haddad et al., 2019). In higher prevalence countries, rates in fertility clinics were found to be 1.7% (HIV), 79% (HBV) and 0.4% (HCV), similar to reported rates in their general populations (Yakass et al., 2016).

The frequency of testing has been considered in other jurisdictions and in other related scenarios. Based on aforementioned research and other scientific and economic factors, and in keeping with European Union regulations, the UK and Human Fertilisation and Embryology Authority (HFEA) have determined that testing every 24 months is not associated with additional risk of cross-contamination in the laboratory compared with yearly testing.

Another example of management of cross-contamination risk is that between healthcare workers and patients. There is no routine recommendation for testing of healthcare workers for BBVI to prevent patient contamination. In almost every Canadian province, there is no testing of physicians who perform

**TABLE 7** INDICATIONS FOR ANNUAL OR BIANNUAL BBVI TESTING

HIV risk factors	HCV risk factors	HBV risk factors
Annual testing		
Persons with multiple sexual partners A diagnosis of other sexually transmitted infection Received healthcare or personal services where there is a lack of infection prevention and control practices	A diagnosis of other sexually transmitted infection Received healthcare or personal services where there is a lack of infection prevention and control practices History of recent or current incarceration Having haemodialysis	Persons with multiple sexual partners A diagnosis of other sexually transmitted infection Received healthcare or personal services where there is a lack of infection prevention and control practices History of recent or current incarceration Having haemodialysis Using immune-modulation therapy or who are immunosuppressed
Biannual testing		
Current injection drug use For men, sex with other men	Current injection drug use For men, sex with other men	Current injection drug use For men sex with other men Using immune modulation therapy or who are immunosuppressed

HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus.

exposure-prone procedures even though infected, undiagnosed surgeons pose a risk to patients through blood-borne contamination. In Ontario, these physicians are required to undergo testing every 3 years to reduce the risk of direct infection. The risk of indirect contamination (as might happen in other medical specialties) is deemed to be so low so as not to warrant testing. This is based on expert consensus and can be assumed to reflect Canadian values.

#### **Recommendations:**

- 25 ART providers should test reproductive partners for HIV serology, HBV surface antigen and HCV antibody at intake.
- 26 ART providers should re-test individuals with any risk factors for these BBVI annually or biannually (**TABLE 7**).
- 27 In the absence of any BBVI risk factors (see **TABLE 7**), ART providers should not re-test for BBVI for the purpose of preventing cross-contamination.

#### **CONCLUSION**

The management of individuals living with BBVI has advanced considerably, leading to improved prognosis and quality of life, and a greater desire to parent. Current practices in ART do not reflect these changes as many ART clinics inappropriately deny services to people living with BBVI. There remain important gaps in the availability of ART to those living with BBVI across Canada, often due to the unwarranted fears of those providing care.

The goal of this CFAS Clinical Practice Guideline is to address two important considerations with regard to BBVI in the ART setting: (i) managing male-to-female transmission of BBVI, and (ii) preventing cross-contamination. This guideline interprets the best available evidence and considers the needs of the patient, the care providers and society. Individuals properly treated for HIV and HCV pose no infection risk to their partners or other patients using assisted reproduction. Laboratories can simply and effectively mitigate risk from individuals with active BBVI by individual preparation of gametes. Embryos in culture or in storage pose no threat in the absence of equipment failure and likely pose no threat even in the event of failure. The risk of both transmission and cross-contamination can effectively be eliminated by any and all ART

laboratories while providing care to all who need it. Ultimately, the practices in this guideline are best practices for all ART patients regardless of BBVI status and we hope this guideline will expand ART access to people living with BBVI on the basis of the best available evidence.

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