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Appendix 2. Detection and Risk Modeling

1. General background: blood screening and viral dynamics

Current blood screening reliably tests for HIV RNA and anti-HIV antibodies

In the U.S., units of donated blood are screened for HIV-1/2-targeting antibodies (serological testing) and HIV-1 RNA (nucleic acid testing or NAT) [1]. Serological testing is performed on individual samples, using a chemiluminescence immunoassay that detects antibodies against HIV-1 and antibodies against HIV-2 (Abbott PRISM HIV O Plus, in use since 2010) [2]. Due to cost and logistical reasons, NAT is typically performed on pools of 16 samples (mini-pool NAT or “MP-NAT”). The assay employs transcription-mediated amplification (TMA) and a hybridization protection assay (HPA) to detect HIV-1 RNA in infected samples (Procleix Ultrio Plus, used since 2013) [3].¹ Although common in clinical settings, screening for HIV antigens was largely abandoned for blood products after the introduction of more sensitive NAT methods in 1999 and subsequent FDA support. (In 2002, the FDA licensed NAT testing for commercial sale and issued draft guidance on the use of NAT to reduce the risk of HIV transmission.)

Screening algorithm

Donations with reactive test results (NAT and/or ChLIA) are subject to confirmatory testing and, if found repeat reactive, discriminatory testing to distinguish pathogens (triplex tests for HIV, HBV, and HCV are often used for initial screening) and HIV subtype [1, 4, 5]. Donors of reactive samples are notified and counseled based on their test results.

Viral dynamics and test window periods

Blood tests differ in how early they can detect an HIV infection after transmission [6-8]. Besides individual test sensitivity, this “window period” largely depends on the dynamics of an HIV infection. The levels of biomarkers commonly used for detection—viral RNA and anti-HIV antibodies—follow a characteristic temporal pattern (**Figure 1**).²

Following transmission, the virus is thought to first replicate locally at the site of infection without entering the bloodstream (“pre-viremia period”) [9]. Following the establishment of initial viremia, viral loads quickly grow to detectable levels in a matter of days. The total infection-to-detection period (ITD), often referred to as the “eclipse period”, has been estimated to last between 7 and 21 days (mean 10 days) [9]. While cases of significantly longer ITD periods have been described, they seem to be rare [10].

Following the ITD period, HIV blood levels continue their rapid increase to reach peak (21 to 28 days after infection) before dropping again to a relatively stable “virus set point” (**Figure 1**) [6, 8, 10-12]. Antibodies against HIV usually reach detectable levels around 22 days,

¹ Data from the American Red Cross (ARC), Blood Systems, Inc. and the New York Blood Center, which together handle approximately 50% of the US blood supply.

² The detection of HIV antigens is currently not used to screen the majority of US blood donations.

the time of peak viremia. This dynamic of acute HIV infections is highly consistent between subjects and is the main reason for window-period differences between specific types of blood tests such as serological testing and NAT.

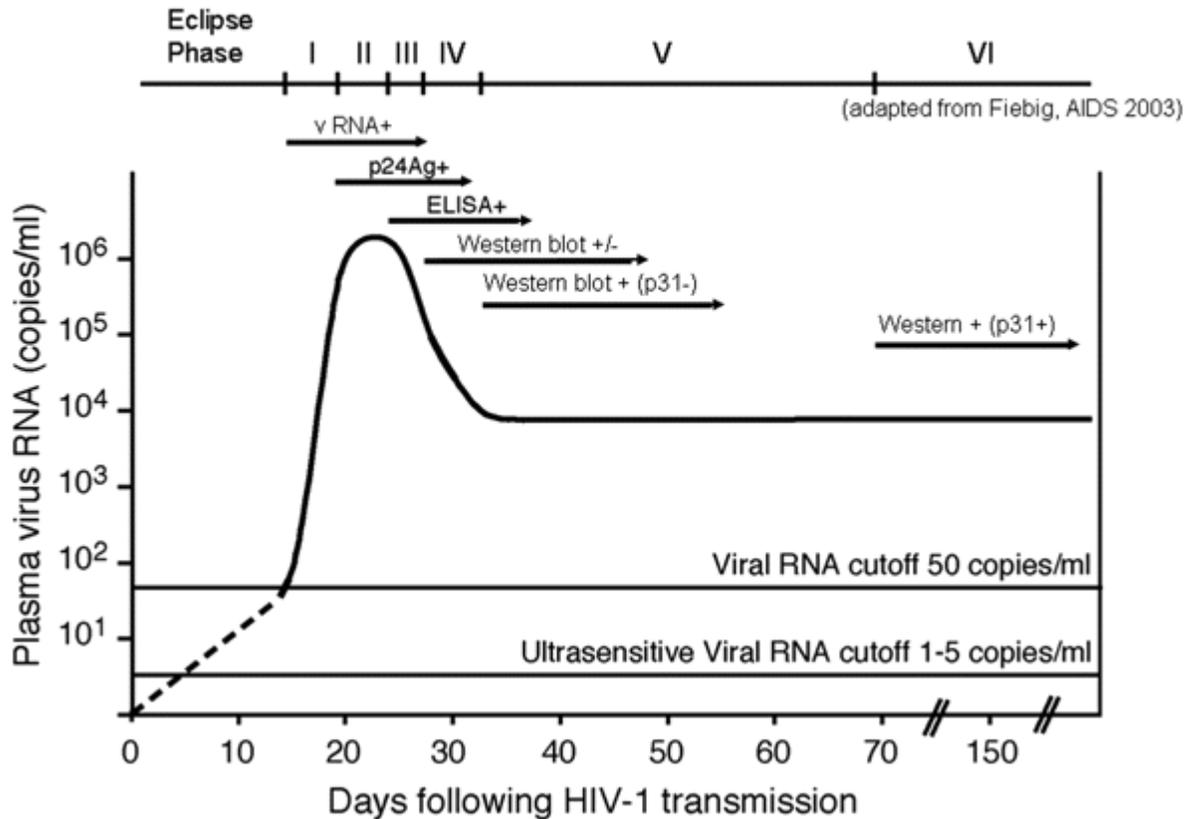


Figure 1. Natural History and Laboratory Staging of HIV Infection (from [9]).

While the relative window-period differences between tests can be accurately measured [7], variability in and lack of data on the length of the pre-viremia period make absolute quantification of window periods difficult [9, 10]. Studies on cases with known exposure dates indicate, however, that the majority of people reach NAT-detectable viremia within 15 days [13].

For risk calculations, however, only the period of time in which an *infectious* sample escapes detection is of interest. Absolute window periods are therefore estimated as the time between reaching *infectious viremia* and detection (thus excluding the variable pre-viremia period). Consistent HIV growth rates have been observed across subjects during early infection, which allow back-propagating viral copy numbers from the lower limit of detection to infectious viremia (estimated to be 1 copy in 20 mL based on the conservative assumption that even one copy in a 20 mL transfusion unit would be infectious) [7].

Based on this model, individual nucleic acid testing (ID-NAT) is expected to detect infectious viremia after 5.6 days, mini-pool NAT (MP-NAT) after 9 days, p24 antigen testing after 15 days, and antibody detection after 20.3 days (**Figure 1**) [6-8]. NATs can thus detect an

infection several days earlier than other tests. This reduction in window period is attributed with the lower risk of HIV transmissions observed since the introduction of NAT in 1999 [14].

2. Risk assessment

Risk under a lifetime deferral

Current estimates place the residual risk for releasing an HIV-infected blood unit at 1 in 1,467,000 [14], or 9-10 blood units out of a total 14 million in the United States per year. These estimates are based on statistics from 1999 to 2008. They therefore reflect risk under a lifetime ban for MSM (which was in place until 2015, before being shortened to 12 months).

Estimated risk for periods exceeding test window periods

Anderson *et al.* used a probabilistic model to estimate risk increase under a 1-year or 5-year deferral policy [17]. These periods are significantly longer than blood test window periods as well as the assumed time from transmission to infectious viremia [9, 10]. Therefore, the model estimates risk increases based on false-negative testing subsequent to the window period as well as logistic errors (e.g. erroneously releasing quarantined samples). The study reported extremely low increases in risk of 0.5% (5-year deferral) and 3% (1-year deferral), indicating that false-negative testing subsequent to the NAT and antibody test window period and logistic errors lead to only minor increases in risk. This is expected given the extremely high sensitivity of HIV testing (>99.8% for both NAT and antibody testing) and the continued reduction in logistic errors due to automation [17]. We thus focus only on window-period donations when calculating the risk for shorter deferral periods in the following sections and the main text.

Estimated risk for complete lift of deferrals

In 2015, Yang *et al.* estimated that complete removal of risk assessment questions (*i.e.*, risk-based deferrals) could lead to an additional 31 units of HIV-infected blood from MSM entering the blood supply, increasing the risk 3-fold to about 1 in 340,000 [15]. This model focused entirely on risk associated with window-period transmissions, which would make up the vast majority of cases under this scenario (false-negative tests outside the window period and logistic errors are extremely unlikely by comparison).

As stated by the authors, these numbers represent a “worst-case scenario” [15], assuming that individual risk assessment is either not performed or is completely ineffective. In addition, the worst-case model is based on the assumption that MSM donate regardless of self-perceived risk, *i.e.* 0% self-deferral. Modeling a self-deferral rate of 75% (50%) among MSMs would lower the risk to 8 (16) additional infected units per year or 1 in 780,000 (1 in 540,000) [15]. A recent study indicates that this is a more realistic scenario [1]. The HIV prevalence among noncompliant MSM (men who donated blood despite MSM behavior) could be calculated as 0.16%, indicating a 3- to 6-fold reduction over the general MSM population (0.522% - 0.989%) [16], *i.e.* a 69-84% self-deferral rate.

3. Estimated risk for shortening of the deferral period

Predicted effects of shorter deferrals on the number of infected units of blood

We followed the Yang *et al.* model to estimate window-period risk for deferral periods shorter than one year. Based on this model, reducing the deferral time to ~15 days is expected to lead to only very small increases in residual risk (< 20%; see main text for details).

Predicted effects of shorter deferrals on the number of cases of HIV transmission by transfusion

Applying a window-period model (like the Yang *et al.* model) to the current residual risk estimate predicts 10.2 infected units per year. This estimate is much higher than the number of observed transfusion-transmitted HIV cases over the last decade (since 2003, only one case of HIV-1 transmission through blood products has been observed in 2008) [18].

It is possible that some cases of HIV infection in patients have gone unobserved, for instance, if the patients died shortly after transfusion and were never tested for HIV. A 2004 U.S. study found that 46% of transfused patients were alive after 5 years [19]. Similar numbers were found in England (46.9%; [20]) and Denmark and Sweden (53.4%; [21]). A lower estimate of 32% was found by Dorsey *et al.* [22]. In addition, there is evidence that the common practice of leukoreduction may lower risk of HIV transmission from infected blood (additional details on leukoreduction can be found in Appendix 4).

Evidence from other countries supports the hypothesis that the Yang *et al.* model overestimates the number of transmission: four other nations that have recently relaxed their deferral policies have seen no subsequent rise in HIV infections.

- In Spain, the policy was changed to individual risk assessment (IRA) in 2005. Following the change, there have been no documented instances of HIV transmission by donated blood products. There have been no cases of HIV transmission by blood in Spain since 2005 [23].
- In Italy, the policy was changed to IRA in 2001. The last case of transfusion-transmitted HIV infection in Italy was reported in 2005 [24].
- In Japan, the policy was changed from 12-month deferral to 6-month deferral in 2011. There have been no incidents of infection through blood transfusion in Japan since the 1980s [25].
- In South Africa, which used to have a 6-month deferral period between 2006 to 2014, there were no reported cases of HIV transmission through blood transfusion over the last 8.5 years [26]. There are no public data available to show any change in overall HIV transmission rates after policy was changed to no-deferral in 2014.

We note that Italy and Spain employ in-person interviews with trained healthcare professionals who decide whether to apply temporary (4-12 months) or permanent deferral to donors who report potentially risky sexual practices, such as having new or casual partners whose HIV-status

and sexual behavior is unknown or risky (e.g., they frequently change partners). Recurring sex with multiple casual partners, for example, is grounds for deferral of any donor in Italy [24].

4. References

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Appendix 3. Individualized Risk Assessment

1. Temporary donor deferral

Blood donor screening has long relied on a donor history questionnaire (Supplementary 1) to assess individualized risk for transfusion-transmissible infections and thus reduce the number of infected units that enter the biochemical screening pipeline. Current FDA guidelines [1] recommend lifetime deferral for candidate donors who report a history of injection drug use or sex work, whereas candidates reporting typical sexual risk factors for acquiring HIV, such as an HIV-positive sex partner, are temporarily deferred for 12 months from the precipitating event.

The purpose of temporary deferral³ is to prevent donations by individuals who have been recently infected and might not yet be identified through biochemical screening (“window-period” donations). As shown in Appendix 2, scientific consensus on HIV infection dynamics and detection suggests a short period of 2-3 weeks would serve the same purpose as a 12-month deferral with comparably low risk. Under this assumption, the goal of individualized risk assessment is to defer candidates whose behavior in the preceding few weeks puts them at risk of being in the window period.

2. Temporary deferral for all sexually-active MSM

Currently, all sexually-active MSM are deemed at risk of acquiring HIV and thus subject to temporary deferral. While MSM who do not report injection drug use account for roughly 67% of annual HIV diagnoses ([2]; **Figure 1**), a strong body of evidence exists to suggest not all sexually-active MSM are equally at risk. Automatic assignment of high-risk status to all MSM does not take into account the specific behaviors and sexual practices likely to predispose an individual (of any gender or orientation) to acquiring HIV.

³ We focus on deferral due to sexual risk factors for HIV, as this is where MSM and non-MSM donors may differ. Other factors like travel history, general medical history, contact with blood, etc. can be taken to apply equally.

**New HIV Diagnoses by Transmission Category
(2015, n=39,513)**

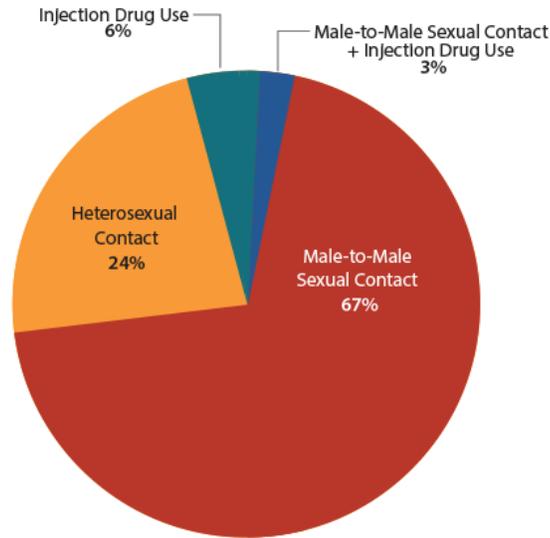


Figure 1. New HIV diagnoses by transmission category, 2015 [3].

Unprotected receptive anal intercourse carries the highest risk of transmission, estimated on average at 1.4% per viral exposure, while other practices are orders of magnitude less risky ([4-6]; **Figure 2**). Behaviors such as avoiding anal intercourse, proper use of condom protection during anal intercourse, having a small number of sexual partners, and being in a relationship with an HIV-negative partner, are all associated with significantly reduced risk of infection [7-11].

Type of exposure	Estimated per-act probability of acquiring HIV
Receptive anal intercourse	1/72
Insertive anal intercourse	1/910
Receptive vaginal intercourse	1/1,250
Insertive vaginal intercourse	1/2,500
Oral intercourse	Low

Figure 2. Estimated per-act probability of acquiring HIV, by exposure act [6].

Three surveys conducted among representative samples of MSM in California demonstrated that a large proportion do adhere to safer sexual practices. These studies suggested roughly 60-70% of MSM, most of whom are currently deferred, may reliably be at low risk of HIV since they either had no or few sexual partners in the preceding 12 months, avoided anal intercourse, or consistently practiced condom protection ([12, 13]; **Figure 3**).

Sexual practice	Urban Men’s Health Study, San Francisco, 1997 (n = 915)	Urban Men’s Health Study, San Francisco, 2002 (n = 879)	California Health Interview Survey, 2002 (n = 398)	Number of sexual partners	California Health Interview Survey, 2002 (n = 398)
No male sexual contact	14.8%	13.5%	10%	0	10%
No anal intercourse	25%	18.1%	24%	1	32%
100% condom-protected anal intercourse	32.1%	27%	28%	2-4	29%
Unprotected anal intercourse	28.1%	41.4%	38%	5 or more	29%

Figure 3. Self-reported sexual behavior over the past 12 months in three probability telephone surveys of MSM in California [12, 13].

Similarly, the 2014 National HIV Behavioral Surveillance study [14] of nearly 10,000 sexually-active MSM in 20 U.S. cities (80% of whom were HIV-negative) found that a third had refrained from unprotected anal intercourse in the preceding 12 months. Furthermore, 45% of those who engaged in unprotected intercourse did so only with a main partner,⁴ as opposed to casual partners. The median number of sexual partners over 12 months was 4, which is below the number usually associated in studies with elevated risk of infection (10 or more).

This study also asked participants about their last sexual encounter, which is much more predictive of recent infection that could lead to window-period donations. Importantly, 25% reported no anal intercourse, 34% engaged in condom-protected intercourse, 17% engaged in unprotected intercourse exclusively as the insertive partner, and 24% engaged in the highest risk behavior of unprotected intercourse as the receptive partner (or in both roles).

In addition to the sexual practices directly linked to viral transmission, longitudinal studies have identified correlates of increased risk for acquiring HIV [7, 8, 10, 15]. The most prominent among MSM are drug use (specifically methamphetamines) and a recent diagnosis of

⁴ A main partner was defined in the study as: “a person with whom the participant has sex and to whom he feels most committed (e.g., boyfriend, husband, significant other, or life partner).”

other sexually-transmitted infections. Other correlates, such as age or geographic location, are also associated with risk of infection.

Notably, various combinations of the sexual, behavioral and other characteristics described above are already routinely used to identify MSM at low or high risk of HIV infection for recruitment to HIV-vaccine clinical trials (Supplementary 2), or by clinicians evaluating MSM patients for testing and preventive interventions ([10]; Supplementary 3).

At the same time, non-MSM individuals (men and women) who report no injection drug use still account for 24% of annual HIV diagnoses (Figure 1) and might not currently be identified and deferred, emphasizing that the risk of window period donation is not limited to MSM.

It therefore seems clear that deferral of all sexually-active MSM excludes many potential donors who are at low risk and who can likely be identified by appropriate criteria; and that some at-risk non-MSM donors are not presently deferred. This suggests both the safety of the blood supply and the fairness of blood donation policy would benefit from an individualized risk assessment.

3. Screening algorithm to identify donors at low-risk of recent infection

The donor history questionnaire can be amended to include questions that identify MSM donors who are at low risk of HIV infection through sexual contact in the preceding 2-3 weeks. Some or all of these questions could potentially be presented to non-MSM donors as well,⁵ though the risk assessment based on their answers would likely differ. Thus, in any case, candidate donors should be asked to indicate whether they are MSM.

Below we illustrate an example of a possible screening algorithm and a minimal set of potential questions to solicit the necessary information.

Step 1: Apply current deferral criteria

The criteria already used to temporarily defer donors due to risk of sexual exposure to HIV (besides MSM behavior) address clear risk factors for donors of any gender or sexual orientation.

In the past 2-3 weeks:

- a) Have you had sexual contact with a partner you know is HIV-positive, or at high risk of exposure to HIV (injection drug user, sex worker)?
Yes/No
- b) Have you been diagnosed or treated for a sexually-transmitted infection (chlamydia, gonorrhea, syphilis)?
Yes/No

⁵ Specifically, unprotected anal/vaginal intercourse with a large number of partners is a risk factor for non-MSM.

Step 2: Individualized risk assessment based on recent sexual history

Candidate donors not deferred in Step 1 would be evaluated based on their recent sexual behavior. Two complementary approaches can be taken to define low-risk behavior: i) donors who are unlikely to have had an HIV-positive sex partner, and ii) donors who are unlikely to have contracted HIV based on their sexual practices and protective measures.

In the past 2-3 weeks:

- a) Have you had sexual contact with a partner in a long-term relationship (>6 months)?

Yes/No

If yes:

- (i) To the best of your knowledge, has your long-term partner been tested for HIV in the last 6 months and found not to be infected (HIV-negative)?

Yes/No/Not sure

- (ii) To the best of your knowledge, has your long-term partner had sexual contact in the last 6 months with anyone other than you?

Yes/No/Not sure

- b) Have you had sexual contact with a new partner (<6 months), or with casual partners?

Yes/No

If yes:

- (i) With how many partners have you had sexual contact?

1/2/3 or more

- (ii) Have you had, on any occasion, anal intercourse without proper use of a condom?

Yes/No

- (iii) Have you had, on any occasion, sexual contact under the influence of drugs such as speed or crystal meth?

Yes/No

In addition, it may be useful to include a question about pre-exposure prophylaxis (PrEP) treatment to improve donor compliance. Some individuals who are on PrEP and perceive themselves to be at very low risk of infection might be tempted to misrepresent risky practices to accord them with their self-perception. Allowing donors to expressly state they are on PrEP treatment could potentially prevent such behavior.

MSM donors would thus be deferred only if they reported: sex with a long-term partner who has not recently tested HIV-negative, unprotected anal intercourse with any new/casual partner, >1-2 new/casual partners overall, or sex under the influence of drugs⁶ with new/casual partners. The precise parameters and conditions of the risk assessment eventually adopted should

⁶ The use of methamphetamines is consistently correlated with the risk of HIV infection in longitudinal studies of MSM. It is reasonable to suspect that sexual acts under the influence of these drugs might not be recalled or reported accurately.

be based on the best available epidemiological data and could also consider age, gender or location.

4. Questionnaire administration

Current methods for administering the questionnaire are at the discretion of each collection center, and include face-to-face, computerized or a combination of both. A retrospective study of HIV-positive blood donors in Australia indicated that noncompliance could be significantly reduced through the use of ACASI (Audio Computer-Assisted Self-Interview), which provides a greater degree of privacy and comfort [16]. Similarly, studies at American Red Cross blood donor centers have also demonstrated that ACASI elicits more accurate and honest responses to questions that predict risk of HIV [17]. The Red Cross has started using the RapidPass system⁷ to allow candidate donors to complete and print the questionnaire at home on the day of donation. At the donation site, a staff member then reviews the questionnaire and conducts follow-up procedures. More universal application of this methodology can reasonably be expected to increase donor compliance and blood safety.

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⁷ <http://www.redcrossblood.org/rapidpass>

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Supplementary 1. Current recommended full-length donor history questionnaire in the United States

Full-Length Donor History Questionnaire (DHQ)

	Yes	No
Are you		
1. Feeling healthy and well today?	<input type="checkbox"/>	<input type="checkbox"/>
2. Currently taking an antibiotic?	<input type="checkbox"/>	<input type="checkbox"/>
3. Currently taking any other medication for an infection?	<input type="checkbox"/>	<input type="checkbox"/>
4. Have you taken any medications on the Medication Deferral List in the time frames indicated? (Review the Medication Deferral List.)	<input type="checkbox"/>	<input type="checkbox"/>
5. Have you read the educational materials today?	<input type="checkbox"/>	<input type="checkbox"/>
In the past 48 hours,		
6. Have you taken aspirin or anything that has aspirin in it?	<input type="checkbox"/>	<input type="checkbox"/>
In the past 8 weeks, have you		
7. Donated blood, platelets or plasma?	<input type="checkbox"/>	<input type="checkbox"/>
8. Had any vaccinations or other shots?	<input type="checkbox"/>	<input type="checkbox"/>
9. Had contact with someone who was vaccinated for smallpox in the past 8 weeks?	<input type="checkbox"/>	<input type="checkbox"/>
In the past 16 weeks,		
10. Have you donated a double unit of red cells using an apheresis machine?	<input type="checkbox"/>	<input type="checkbox"/>
In the past 12 months, have you		
11. Had a blood transfusion?	<input type="checkbox"/>	<input type="checkbox"/>
12. Had a transplant such as organ, tissue, or bone marrow?	<input type="checkbox"/>	<input type="checkbox"/>
13. Had a graft such as bone or skin?	<input type="checkbox"/>	<input type="checkbox"/>
14. Come into contact with someone else's blood?	<input type="checkbox"/>	<input type="checkbox"/>
15. Had an accidental needle-stick?	<input type="checkbox"/>	<input type="checkbox"/>
16. Had sexual contact with anyone who has HIV/AIDS or has had a positive test for the HIV/AIDS virus?	<input type="checkbox"/>	<input type="checkbox"/>
17. Had sexual contact with a prostitute or anyone else who takes money or drugs or other payment for sex?	<input type="checkbox"/>	<input type="checkbox"/>
18. Had sexual contact with anyone who has ever used needles to take drugs or steroids, or anything <u>not</u> prescribed by their doctor?	<input type="checkbox"/>	<input type="checkbox"/>
19. Male donors: Had sexual contact with another male?	<input type="checkbox"/>	<input type="checkbox"/>
20. Female donors: Had sexual contact with a male who had sexual contact with another male in the past 12 months?	<input type="checkbox"/>	<input type="checkbox"/>
21. Had sexual contact with a person who has hepatitis?	<input type="checkbox"/>	<input type="checkbox"/>
22. Lived with a person who has hepatitis?	<input type="checkbox"/>	<input type="checkbox"/>
23. Had a tattoo?	<input type="checkbox"/>	<input type="checkbox"/>
24. Had ear or body piercing?	<input type="checkbox"/>	<input type="checkbox"/>
25. Had or been treated for syphilis or gonorrhea?	<input type="checkbox"/>	<input type="checkbox"/>
26. Been in juvenile detention, lockup, jail, or prison for more than 72 consecutive hours?	<input type="checkbox"/>	<input type="checkbox"/>

In the past three years, have you		
27. Been outside the United States or Canada?	<input type="checkbox"/>	<input type="checkbox"/>
From 1980 through 1996,		
28. Did you spend time that adds up to 3 months or more in the United Kingdom? (Review list of countries in the UK)	<input type="checkbox"/>	<input type="checkbox"/>
29. Were you a member of the U.S. military, a civilian military employee, or a dependent of a member of the U.S. military?	<input type="checkbox"/>	<input type="checkbox"/>
From 1980 to the present, did you		
30. Spend time that adds up to 5 years or more in Europe? (Review list of countries in Europe.)	<input type="checkbox"/>	<input type="checkbox"/>
31. Receive a blood transfusion in the United Kingdom or France? (Review country lists.)	<input type="checkbox"/>	<input type="checkbox"/>
Have you EVER		
32. Female donors: Been pregnant or are you pregnant now?	<input type="checkbox"/>	<input type="checkbox"/>
33. Had a positive test for the HIV/AIDS virus?	<input type="checkbox"/>	<input type="checkbox"/>
34. Used needles to take drugs, steroids, or anything <u>not</u> prescribed by your doctor?	<input type="checkbox"/>	<input type="checkbox"/>
35. Received money, drugs, or other payment for sex?	<input type="checkbox"/>	<input type="checkbox"/>
36. Had malaria?	<input type="checkbox"/>	<input type="checkbox"/>
37. Had Chagas disease?	<input type="checkbox"/>	<input type="checkbox"/>
38. Had babesiosis?	<input type="checkbox"/>	<input type="checkbox"/>
39. Received a dura mater (or brain covering) graft or xenotransplantation product?	<input type="checkbox"/>	<input type="checkbox"/>
40. Had any type of cancer, including leukemia?	<input type="checkbox"/>	<input type="checkbox"/>
41. Had any problems with your heart or lungs?	<input type="checkbox"/>	<input type="checkbox"/>
42. Had a bleeding condition or a blood disease?	<input type="checkbox"/>	<input type="checkbox"/>
43. Have any of your relatives had Creutzfeldt-Jakob disease?	<input type="checkbox"/>	<input type="checkbox"/>

Source: <http://www.aabb.org/tm/questionnaires/Documents/dhq/v2/DHQ%20v2.0.pdf>

Supplementary 2. A sample of HIV-vaccine clinical trial inclusion/exclusion criteria pertaining to risk of acquiring HIV

This study has been completed.

Sponsor:

National Institute of Allergy and Infectious Diseases (NIAID)

Information provided by:

National Institute of Allergy and Infectious Diseases (NIAID)

▶ Eligibility

Ages Eligible for Study: 18 Years to 55 Years (Adult)
Genders Eligible for Study: Both
Accepts Healthy Volunteers: Yes

Criteria

Inclusion Criteria

- HIV negative.
- Acceptable methods of contraception.

Exclusion Criteria

- Identifiable risk behavior for HIV infection, including: sexual partners of HIV positive people, sexual intercourse with a partner of unknown HIV status if that partner is reported to be at higher risk for HIV infection, gay men reporting any unprotected anal intercourse with partners of unknown status in the 12 months preceding study entry, individuals diagnosed with a sexually transmissible infection (STI) in the 12 months preceding entry that may have been acquired through anal or vaginal intercourse, individuals reporting sharing of injecting equipment in the last 12 months.

Source: Excerpted from <https://clinicaltrials.gov/ct2/show/NCT00051454>

ClinicalTrials.gov Identifier:

NCT00051454

First received: January 10, 2003

Last updated: August 23, 2007

Last verified: August 2007

[History of Changes](#)

This study has been completed.

Sponsor:

National Institute of Allergy and Infectious Diseases (NIAID)

Information provided by:

National Institutes of Health Clinical Center (CC)

Criteria

- INCLUSION CRITERIA:

A participant must meet all of the following criteria:

1. 18 to 50 years old.
2. Available for clinical follow-up through Week 24 of the study for subjects in Part I: available for clinical follow-up through Week 52 for subjects in Part II and committed to 4 years of annual follow-up contact after Week 52 if in Part II of the study.
3. Able to provide proof of identity to the satisfaction of the study clinician completing the enrollment process.
4. Complete an Assessment of Understanding (that includes understanding of Step Study results) prior to enrollment and verbalize understanding of all questions answered incorrectly.
5. Able and willing to complete the informed consent process.
6. Willing to receive HIV test results and willing to abide by NIH guidelines for partner notification of positive HIV results.
7. Willing to donate blood for sample storage to be used for future research.
8. Willing to discuss HIV infection risks, amenable to risk reduction counseling, committed to maintaining behavior consistent with low risk of HIV exposure through the last required protocol clinic visit and assessed by the clinic staff as being at low risk of HIV infection on the basis of behaviors in the 12 months prior to enrollment as follows: Sexually abstinent OR Had two or fewer mutually monogamous relationships with partners believed to be HIV-uninfected and who did not use injection drugs, crack cocaine or methamphetamine OR Had three or fewer partners believed to be HIV-uninfected and who did not use injection drugs, crack cocaine or methamphetamine and with whom he/she regularly used condoms for vaginal or anal intercourse.

Source: Excerpted from <https://clinicaltrials.gov/ct2/show/NCT00479999>

ClinicalTrials.gov Identifier:

NCT00479999

First received: May 26, 2007

Last updated: January 29, 2015

Last verified: May 2014

[History of Changes](#)

Supplementary 3. MSM HIV risk calculators currently recommended in clinical practice based on predictive sexual, behavioral and other factors

a)

MSM Risk Index ²⁵		
1	How old are you today?	If <18 years, score 0 If 18-28 years, score 8 If 29-40 years, score 5 If 41-48 years, score 2 If 49 years or more, score 0
2	In the last 6 months, how many men have you had sex with?	If >10 male partners, score 7 If 6-10 male partners, score 4 If 0-5 male partners, score 0
3	In the last 6 months, how many times did you have receptive anal sex (you were the bottom) with a man without a condom?	If 1 or more times, score 10 If 0 times, score 0
4	In the last 6 months, how many of your male sex partners were HIV-positive?	If >1 positive partner, score 8 If 1 positive partner, score 4 If <1 positive partner, score 0
5	In the last 6 months, how many times did you have insertive anal sex (you were the top) without a condom with a man who was HIV-positive?	If 5 or more times, score 6 If 0 times, score 0
6	In the last 6 months, have you used methamphetamines such as crystal or speed?	If yes, score 6 If no, score 0
		Add down entries in right column to calculate total score
		TOTAL SCORE*

* If score is 10 or greater, evaluate for intensive HIV prevention services including PrEP. If score is below 10, provide indicated standard HIV prevention services.

Source: <http://www.cdc.gov/hiv/pdf/PrEPProviderSupplement2014.pdf>, Smith, D.K., *et al.*, *Development of a clinical screening index predictive of incident HIV infection among men who have sex with men in the United States*. J Acquir Immune Defic Syndr, 2012. **60**(4): p. 421-7.

b)

Does your patient/client have gonorrhea, chlamydia, or syphilis, or does he have a history of these infections?	If yes, add 4 points If no, add 0 points	_____
Has your patient/client used methamphetamine or inhaled nitrites (poppers) in the prior 6 months?	If yes, add 11 points If no, add 0 points	_____
Does your patient/client report unprotected anal intercourse with a partner of positive or unknown HIV status in the prior year?	If yes, add 1 point If no, add 0 points	_____
Does your patient/client report 10 or more male sexual partners in the prior year?	If yes, add 3 points If no, add 0 points	_____
Sum total number of points		_____ Total Points

Total Points	Estimated percentage of men with this score who will acquire HIV over 4 years
0	<5%
1-3	5%-9%
4-11	10%-14%
12+	> 14%

How to use the chart

1. Calculate your patient's/client's risk score.
2. Match the risk score with the point range provided on the table to estimate 4-year HIV risk.
3. Follow up with testing recommendations, referrals to services, and prevention intervention according to risk.

Source: Menza, T.W., *et al.*, *Prediction of HIV acquisition among men who have sex with men*. Sex Transm Dis, 2009. **36**(9): p. 547-55.

Appendix 4. Alternative Testing Methods and Leukoreduction

1. Alternative testing methods

Reducing the window period between infectious viremia and reliable detection by blood tests is the most effective way of reducing residual risk [1, 2]. Currently used NAT tests can detect infections after ~9 days, when plasma viral RNA levels exceed 100 copies per mL (cp/mL). Different testing procedures and novel methodologies have been shown to lower the limit of detection to less than 1 cp/mL, which could potentially decrease the window period to less than a day after developing infectious viremia.

Individual nucleic acid testing is more sensitive than pooled testing

NAT testing at the ARC is currently performed in mini-pools of 16 samples (MP-NAT). It has been established that testing samples individually (ID-NAT) is more sensitive than pooled testing [3]. Busch *et al.* estimate that switching from MP-NAT to ID-NAT could decrease the window period by 3.4 days [4].

New testing methods with higher sensitivity can shorten the window period

Newer clinically approved tests already improve sensitivity to 20 copies per mL, thus reducing the estimated window period to 7.4 days (**Table 1**).

The most sensitive test reported to date can detect 0.6 (0.06) copies per mL with a test volume of 3 mL (30 mL), theoretically closing the window period to 3 (0.2) days. Achieving these levels of sensitivity could reduce the current residual risk from 1 in 1.47 million (10.2 window-period units per year) to 1 in 5.5 million (2.5 units per year) or, with the larger-volume version, 1 in 100 million (0.14 units per year).

While these methods need to be validated in and adapted to a clinical setting, they could be used for research on the residual risk associated with window-period donations under a model that discontinues time-based deferral of MSM.

Open questions include the precise dynamics of early HIV infections (below 50 cp/mL) and test volumes. Currently, window-period estimates are based on extrapolating the exponential growth of HIV observed above 50 cp/mL to lower levels of viremia. To make a more accurate prediction, longitudinal studies should be repeated using recently single-copy quantification assays [5]. Another factor to consider is the volume of plasma tested. With sensitivities reaching below 1 cp/mL, it could become a limiting factor to detecting recent infections. We highlight specific testing methods below.

Method	Assay type	Detection limit [cp/mL]	Test volume [mL]	Earliest detection after infectivity [days] ⁸	Residual risk [per 10 ⁶] ⁹	References
Currently used (Procleix Ultrio Plus)	TMA	100	≥ 0.5	9.4	0.56	[7] [8]
Best clinically approved (COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, v2.0)	qRT-PCR	20	1 mL	7.4	0.44	[9]
ddPCR HIV-2 VL ¹⁰	ddPCR	7	unknown	6.1	0.36	[10]
	ddPCR	1	unknown	3.7	0.22	[11]
iSCA	qRT-PCR	0.6	3	3.0	0.18	[12]
Mega-iSCA ¹¹	qRT-PCR	0.06	30	0.2	0.01	[12]

Table 1. Sensitivity, window period, and residual risk for currently available NAT methods.

Single-copy quantification

Improving on the 100 cp/mL sensitivity of the qualitative tests used in blood screening, current commercially available tests are able to quantify viral loads starting at 20 cp/mL [9]. Recently, quantitative real-time PCR (qRT-PCR) assays were developed that are able to detect less than 1 cp/mL. Palmer *et al.* developed an assay targeting HIV-1 *gag* RNA able to quantify samples with 1 cp/mL [13]. This assay was further optimized by Cillo *et al.* by improving RNA extraction and targeting the more conserved HIV-1 *pol* gene, thus achieving a sensitivity of 0.6 cp/mL (assuming 3 mL of plasma are tested) [12]. Sensitivity could be increased by at least 10-fold (0.06 cp/mL) by testing larger plasma volumes of 30 mL.

⁸ Calculated as $(\log_{10}(\text{detection limit}) - \log_{10}(\text{infectivity threshold})) \div (\log_{10} \text{ increase per day})$ according to [6]. We assumed a threshold of infectivity for blood transfusion of 1 viral copy per 20 mL of plasma (practical threshold - a typical RBC transfusion unit contains about 20 mL of plasma) and a \log_{10} -viral-load increase of 0.35 per day.

⁹ Calculated using a window-period model (residual risk = window period * incidence) assuming an incidence of 2.16 per 10⁵ person-years.

¹⁰ This assay was developed for HIV-2.

¹¹ Mega-iSCA is a larger-volume version of iSCA.

ddPCR

Advances in digital PCR technologies and the introduction of droplet digital PCR (ddPCR) show important advantages over the use of qPCR, including the absolute quantification of the nucleic acid sequence of interest and high precision and reproducibility [14]. ddPCR has already been shown to be more accurate in detecting HIV DNA in latent reservoirs and less vulnerable to variations in target sequence or probe concentration [15]. Digital RT-PCR to detect HIV RNA also demonstrates promising results. Ruelle *et al.* were able to establish a detection limit of 7 copies/mL using BioRad's One Step RT-ddPCR kit for HIV-2 infections [10]. Further optimization of protocols is still ongoing to increase sensitivity, while the development of new analysis methods should help decrease the number of false positives [16].

Dual-target NAT

Since 2015, the use of dual-target NAT in Germany has been mandatory as it uses two or more primers to bind and amplify from the HIV genome. This technique results in a reduced risk of NAT false negative tests due to mutations in the primer-binding region of HIV-1 subtype B [17].

2. Leukoreduction

There is evidence that HIV virions are disproportionately concentrated in leukocytes [18]. Leukoreduction, the removal of granulocytes and lymphocytes from blood through filtering, has been shown to reduce infectivity of HIV-infected blood by more than 2 orders of magnitude when applied to blood from HIV-positive donors, in a tissue-culture experiment [19]. Currently, at least 20 countries mandate leukoreduction for all donated blood. Leukoreduction is currently applied to the majority of units donated in the U.S. It can be performed at or soon after initial donation, or by the patient's bedside. The estimated additional cost per unit for universal leukoreduction in the U.S. is \$30 [20], which is feasible for many collection centers to implement. This step does not require significant technical expertise and has been proven to drastically improve blood safety [21]. However, HIV occurs in both cell-associated and cell-free form in infected blood samples and cell-free virus has been shown to be infectious through blood transfusion. The effects on HIV are therefore less pronounced than for viruses that are mostly found to be associated with leukocytes (e.g. cytomegalovirus).

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