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Reassessment of the Diagnostics Window Period for HIV Diagnostics

Work Group 2 Diagnostics & Laboratory HIV & STI*, 29 August 2018

1. Summary

In Switzerland the officially recommended window period for the detection of an HIV infection by serological HIV screening is 12 weeks after exposure. At the time this recommendation was set, the decision for the duration of this period was guided by a strong wish not to miss a single HIV infection, and by lack of data on the seroconversion times with 4th generation tests. Over the last decade HIV screening assays have significantly improved in detecting HIV, rapid tests have entered the market, and more information on the seroconversion kinetics during acute HIV infection accumulated. In this document, working group 2 compiled information on the early biology of HIV infection, the immune response mounted against HIV, and the performance characteristics of available serological diagnostic tests. All of these variables have to be taken into account when deciding about adjusting the window period in revised recommendations.

2. Introduction

Infection with human immunodeficiency virus (HIV) is characterized by a series of early events that lead to the establishment of a definitive persistence of the virus in the human body (1). In order to optimize patient care after assumed exposure to the virus, diagnostic tools have been developed with the aim to reliably detect viral infection in a rapid and reliable way and as early as possible after the event. However, diagnostics in the first weeks after the initial infection event may be challenging as, in most cases, the virus is present only in very small amounts (low inoculation titer). Accordingly, this can lead to a considerable delay until the development of a significant and measurable antibody response. Therefore, for any diagnostic test, the time referred to as “window period” ends when the biomarker, indicating a viral infection, is consistently detected in a suitable diagnostic specimen. Typically, the duration of the window period depends on characteristics of the virus, on the patient’s immune response as well as on technical aspects of the tests. The window periods for tests based on the detection of viral antigens, i.e. capsid protein p24 of HIV-1, or virion RNA are shorter compared to systems detecting patients’ specific antibodies to HIV(2). Shortening these window periods will allow for persons exposed to HIV a quicker response, care and treatment. In addition, such improvement would also shorten the period of anxiety while waiting for the diagnostic result after a risk situation.

* Work Group 2 Diagnostics & Laboratory HIV & STI: PD Dr. med. vet. Jürg Böni, Nationales Referenzzentrum für Retroviren, Zürich; Dr. FAMH Gladys Martinetti, Servizio di microbiologia EOLAB, Bellinzona; PD Dr. phil. nat. FAMH Christoph Niederhauser, Interregionale Blutspende SRK, Bern; Dr ès sc. FAMH Corinne Andreutti, Clinique de la Source, Lausanne; Dr. phil.nat. Franziska Suter-Riniker, Institut für Infektionskrankheiten der Universität Bern, Bern; Dr. Alexander Lüthi, Institut für Infektionskrankheiten der Universität Bern, Bern; Prof. Dr. Thomas Klimkait, Abt. Infektionsdiagnostik, Universität Basel, Basel; Prof. Dr Hans H. Hirsch, FAMH, Abt. Infektionsdiagnostik, Universität Basel, Basel; Dr. sc. nat. FAMH Maya Vonow-Eisenring, Klinik für Immunologie DIA, Universitätsspital Zürich, Zürich; Dr. Sabine Yerly, Laboratoire de virologie, Hôpitaux Universitaires de Genève, Genève; Dr. FAMH Matthieu Perreau, Service d'immunologie et d'allergie CHUV, Lausanne; Dr. FAMH Viviana Rossi, Synlab Labor Dr. Güntert AG, Luzern; Dr. sc. nat. ETH FAMH Diana Ciardo, Viollier AG, Allschwil; Dr. rer. nat. FAMH Günter Dollenmaier, Zentrum für Labormedizin St. Gallen, St. Gallen.

Avoiding a definitive negative HIV test result to a person whose infection would be at the most infectious stages (since viral load would be maximal in this phase) is thus of great importance. According to current recommendations, a negative screening test at week 12 week after possible exposure excludes an HIV infection. In addition, and based on newly available diagnostic tests with higher sensitivity, many European countries have recently changed their policies by shortening the window period and adjusting it to specific conditions.

In this document working group 2 analysed aspects of the biology of HIV and of the immune response to HIV infection, but also the performance characteristics of available diagnostic tests with the aim of assisting the Federal Commission for Sexual Health (EKSG) in the implementation of updated testing rules.

3. The available diagnostic tools

Diagnosis of HIV infection relies on four basic types of diagnostic blood tests, each of which assessing different biological parameters:

- the development of antibodies directed against different specific HIV antigens in serum,
- the presence of viral capsid protein, i.e. p24 antigen of HIV-1 in serum,
- the presence of viral genomic RNA in blood, and
- the presence of proviral HIV DNA in peripheral blood mononuclear cells.

For each of these parameters many diagnostic tests are commercially available. Manufacturing, distribution, and application of in-vitro diagnostic tests in Switzerland is regulated by the Medical Devices Ordinance (SR 812.213), which further specifies that EU guideline 98/79/EC (3) must be fulfilled. Fulfilment of the requirements allows the manufacturer to CE-mark the product.

In vitro diagnostic devices for HIV are included in Annex II, list A of this EU guideline, indicating that these are tests with common technical requirements that are specified by the regulatory authorities. These requirements define the minimal performance these tests must meet in order to be marketed. For certifying the performances of the in-vitro diagnostic tests of list A an independent third party, the so called „Notified body“, has to be involved.

As the initial diagnostic screening for HIV infection in Switzerland and elsewhere relies exclusively on serologic tests, the performance of just these in-vitro diagnostic tests is relevant for the re-assessment of the window period. Screening tests in use can be differentiated in 4th generation assays detecting HIV-1 and HIV-2 antibodies as well as HIV-1 p24 antigen and which are used by all laboratories in Switzerland and in 3rd generation assays, which detects antibodies against HIV-1 and HIV-2 only and which still are used as rapid tests by general practitioners. A CE-marked, combined HIV screening test of the 4th generation, which detects antibodies against HIV-1 and HIV-2 and the capsid antigen p24 of HIV-1 must achieve:

- a sensitivity of ≥ 99%
- a specificity of ≥ 99.5%

- a detection limit of ≤ 2 IU/mL for HIV-1 p24 antigen

EU guideline 98/79/EC also specifies the minimal requirements for rapid HIV tests used by medical professionals or VCT testing sites. Except for a slightly lower specificity of ≥99%, the same criteria apply as for in-vitro laboratory tests.

Consumer products for HIV self diagnosis, i.e. HIV home tests, are also regulated by EU guideline 98/79/EC. HIV consumer tests can therefore be CE-marked and have to meet the same requirements for sensitivity and specificity as the tests for professional use. In addition their suitability for self-testing by lay persons has to be documented.

Currently (date of August 2018), at least these four HIV-1 home tests have received CE-marking, all of them are 3rd generation HIV assays and detect exclusively HIV-specific antibodies:

- INSTI HIV Self Test (bioLytical Laboratories)
- BioSure HIV Self test (BioSure)
- Autotest VIH (AAZlabs)
- Atomo HIV Self Test (atomo diagnostics)

Of note, in Switzerland, the in-house manufacturing and use of diagnostic laboratory devices for HIV infections is legally possible. In order to avoid that such tests undermine the high quality requirements, they have to meet the *„Common technical requirements“* of list A according to EU guideline 98/79/EC as well. Accordingly, the HIV laboratory test concept explicitly states that only CE marked tests can be used for HIV screening, and to our knowledge, no in-house test is currently used for this purpose in Switzerland.

4. Timing early diagnostic events and seroconversion kinetics

Initial events during acute HIV infection

Delineating early events in an acute HIV infection has been difficult to study in HIV-infected individuals for several reasons, including difficulties in diagnosing and obtaining tissue samples very early after HIV infection. For this reason, most studies addressing the key events in acute HIV infection have relied on rhesus macaque models following intravaginal transmission of Simian immunodeficiency virus (SIV)(4). Events during acute HIV infection are typically classified based on the presence or absence of specific virological and/or immunological parameters including HIV genome (HIV RNA) quantification in blood and/or tissues, and the detection of HIV-1 capsid protein p24 and HIV-1 + -2-specific immunoglobulin IgM and/or IgG directed against HIV proteins, i.e. gp120, gp41,p17,p31 and p24 in blood (Reviewed in (5)).

Fiebig Classification of events in Acute HIV infection:

Clinically, early stages of HIV infection can be divided into the stepwise gain in positivity for the detection of HIV antigen p24 and HIV-specific IgM and IgG antibodies, based on which HIV-infected individuals can be categorized into Fiebig stages I–VI (Table 1; (5, 6)). The earliest detectable marker is the

HIV RNA level measured by PCR („viral load“), followed by HIV antigen p24, measured either by enzyme linked immunosorbent assays (ELISA) or Electrochemiluminiscence (ECL) assays; later, the HIV-specific antibodies are detected by ImmuniD-OT-based assays (5).

Eclipse phase

Studies of the intravaginal SIV transmission in rhesus macaque models have revealed that after virus transmission, a period of ~3 days follows, known as the “eclipse phase“, before viral RNA becomes detectable in the plasma (7), while in humans, the eclipse phase can last up to 10 days post-HIV infection (5). During this period, as shown in the monkey model, an enhanced interaction of the transmitted virus with intraepithelial dendritic cells (DCs) or the direct transcytosis may allow to breach the mucosal-epithelial barrier, thereby facilitating the infection of CD4 T cells of the vaginal mucosa (8). At this stage, SIV RNA can be detected by in situ hybridization in endocervical regions (4). Then, following transmission, the virus initially replicates locally in the mucosa before it gets transported to draining lymph nodes, where further amplification occurs. SIV infection then spreads via the thoracic duct and hematogeneously to the lymphatic tissues and the gut (8). By day 7 post-inoculation, SIV-expressing cells are detectable with high frequency in lymph nodes of infected macaques (4); the massive dissemination in lymphoid tissue followed by high levels of virus replication within lymph node-based CD4 T cells possibly account for the burst of viremia that can now be detected in tissues and the circulation. Similarly, HIV viremia peaks between days 7-14 post-infection, reaching 10^6 HIV RNA copies/mL in blood of HIV-infected individuals (9).

Seroconversion window period

The period between HIV infection and the development of detectable HIV-specific antibodies in the serum of patients is referred to as “Seroconversion window period” (5). Seroconversion in most infected individuals normally occurs within 3–4 weeks following infection, at Fiebig stage III, typically when plasma virus loads are reaching their peak. Seroconversion may, however, be delayed in some HIV-infected individuals (discussed below). In practice the term “seroconversion window” refers to time period of the negativity in the HIV screening test. Since the introduction of the 4th generation HIV assays which allow the detection of early HIV-specific IgM/IgG and the HIV-1 viral core antigen p24,.this period includes the ecplipse phase and Fiebig stage I , whereas for HIV-2 infection it extends to Fiebig stage II.

As a consequence of combining the detection of a viral component - HIV-1 p24 antigen - and HIV specific antibodies in a single test, in some infections, a so called “second diagnostic window” can be observed, i.e. there occurs a transient test negativity in the HIV screening assays during the transition from HIV-1 p24 antigen positivity to HIV antibody reactivity. If wrongly dismissed as HIV negative, this phenomenon can delay the diagnosis of an acute HIV infection. Currently, only observational data but no systematic studies are available on this phenomenon and, hence, a thorough estimate of its magnitude and its potential impact on diagnosing HIV infection is not possible.

Table 1: Fiebig stages of Early HIV infection (according to Ref. 6)

Stage	Median Duration	HIV RNA	HIV p24 Ag	Enzyme Immunoassay	Western Blot
Eclipse	11 days	undetectable	undetectable	non-reactive	negative
1	5 days	detectable	undetectable	non-reactive	negative
2	5.3 days	detectable	detectable	non-reactive	negative
3	3.2 days	detectable	detectable	reactive (IgM)	negative
4	5.6 days	detectable	detectable	reactive (IgM)	indeterminate
5	88.6 days	detectable		reactive	positive no p31 band
6		detectable		reactive	positive with p31 band

Current Recommendations and Challenges in the diagnosis of HIV infection

Current diagnostic recommendations suggest that HIV-specific antibody testing detects seroconversion at week 4 post-exposure and provides a good indication of the HIV status, but also points out, based on third-generation HIV-testing, the need for a 12 week waiting period after initial exposure, in order to completely exclude the risk of an HIV infection (10). Recent publications, however, re-analysed the seroconversion kinetics in 1229 individuals, utilizing fourth-generation tests, detecting HIV-specific antibodies (both IgM and IgG) along with the HIV-specific antigen p24. This study showed that window periods ranging around a mean of 20 days (median 18 days) are consistent with previous reports based on mathematical modelling estimates of eclipse period. Yet this time is significantly shorter compared to the estimates based on third-generation antibody tests with an estimated mean of 25 days (median eclipse period of 22 days)(11). The observed window period reduction likely reflects an improved selection of HIV antigen targets as well as the newer amplification chemistries for the signal of the antibody test. In addition, the methods and assumptions used to determine the actual date of seroconversion could also have an impact.

Overall, while it is important to recognize that window periods are only approximations and that there is considerable time variation between individuals, the probability of a false-negative test with current fourth-generation assays is extremely low (0.01) at day 42 (6 weeks) for these tests (11). Accordingly, the table of probabilities of falsely-negative HIV test results (see Table 2) has been suggested to be of utility during pre- and post-test HIV counselling to inform co-decision making regarding the ideal time to test for HIV.

Table 2: **Probability of a negative test result for a third and fourth generation HIV test at various time points in an HIV positive individual (adapted from Taylor et al. ref.11)**

	4th Generation Test			3rd Generation Test		
		Limit of 99% CI Interval			Limit of 99% CI Interval	
Days after Infection	Probability	Lower	Upper	Probability	Lower	Upper
≤9	1.00	0.992	1.000	1.00	0.991	1.000
10	0.99	0.968	0.993	1.00	0.991	1.000
14	0.79	0.748	0.829	0.95	0.920	0.968
22	0.31	0.264	0.355	0.46	0.403	0.509
28	0.08	0.058	0.113	0.13	0.097	0.169
34	0.05	0.034	0.078	0.07	0.049	0.104
42	0.01	0.005	0.027	0.05	0.038	0.088
50	0.00	0.005	0.027	0.05	0.038	0.088
55				0.04	0.028	0.074
65				0.04	0.028	0.074
70				0.03	0.014	0.050
75				0.03	0.014	0.050
80				0.03	0.014	0.050
85				0.01	0.002	0.022
90				0.01	0.002	0.022
95				0.01	0.002	0.022
99				0.00	0.000	0.000

Rapid point-of-care HIV tests have become a valuable tool for HIV prevention and care with a performance comparable to laboratory based tests for the detection of HIV antibodies. However, despite the principal fulfilment of the *common technical requirements* for CE-marking, available rapid tests continue to be less reliable than laboratory-based tests for identifying early HIV infections and require a longer follow-up period before HIV infection can reliably be excluded after a possible exposure. This assessment also applies to combined rapid tests (i.e. 4th generation rapid test) which still have a lower sensitivity for HIV-1 p24 antigen.(12) (13). Dependent on the reference assay used for a comparison

of window period the delay by which 99% of infections are detected by rapid tests is estimated to range from one to two weeks (Table 3).

Table 3: Estimated seroconversion times and window periods (99th percentile) for different serological test types†

	days to reactivity after infection (p = percentile)			
Test Category	**25th p**	**50th p**	**75th p**	**99th p**
antibody/antigen laboratory test	13.0	17.8	23.6	44.3
IgG/IgM-sensitive laboratoy test	18.4	23.1	28.8	49.5
IgG-sensitive rapid test	26.2	31.1	37.0	56.7
Western Blot (IgG sensitive)	31.0	36.5	43.2	64.8

† data according to ref. 14
* note, that the values for Western Blot analyses does not refer to the INNO-LIA used in Switzerland but to the classical tests which is based on viral lysates

5. Modifiers of seroconversion kinetics

Besides variations of the window period that are due to differences in the analytical sensitivity of the various test formats, biological factors have been described which can affect the window period of an infected person by influencing the time course of virus spread or antibody production. They can be attributed to two main categories: exposure to antiretroviral drugs during the early phase of HIV infection, and impaired health conditions that are unrelated to HIV infection.

Exposure to antiretroviral drugs during early infection by post or pre-exposure prophylaxis

Post-exposure prophylaxis (PEP) is given to prevent infection after a suspected HIV exposure. It has been reported that the appearance of viremia and the development of antibodies can be delayed if an HIV infection occurred despite PEP. It is usually recommended to maintain the same follow-up period for PEP as after exposure, i.e. once PEP has been completed.

Pre-exposure prophylaxis (PrEP) is provided to prevent HIV transmission to persons who are at high risk of acquiring HIV by infection. HIV testing should be undertaken every 3-months with a laboratory 4th generation test. Recent results suggest that it may be difficult to diagnose HIV infection in some individuals who continue to take TDF-based PrEP after a transmission event, particularly if less sensitive assays are used for HIV screening. As data on seroconversion under PrEP are only accumulating (15-17) the follow up of persons ceasing PrEP should be dealt with in separate recommendations.

Impact of health and nutritional state on the window period

Substantially delayed HIV-1 seroconversion, defined as positivity by immune blot >12 weeks after exposure, is rare, anecdotal, and was reported in association with one the following conditions:

- Concomitant primary infection or co-infection with another virus (e.g. cytomegalovirus or HCV), which can result in profound immunosuppression and immune system dysregulation (18, 19).
- Malnutrition (19)
- Humoral immunodeficiency related to an MHC haplotype carrying disease susceptibility genes for antibody deficiency disorders, thereby preventing a detectable HIV antibody response (20).
- Initiation of very early antiretroviral therapy (ART) impairing the classical humoral immune response against HIV (21, 22)
- Elite controllers (<1% of untreated patients), who can control the HIV viral load without ART, which results in an atypical and slow development of the antibody response against HIV proteins (23)

Although the time to seroconversion (by HIV immunoblot) may be significantly delayed, the safe diagnosis of an HIV infection can be established in the above-mentioned cases early during infection by demonstrating the presence of p24 antigen and/or viral RNA. This does not apply for some elite controllers, though, in which it can be difficult to prove HIV infection because a of the lack of viral RNA and low levels of proviral DNA in blood However, even among elite controllers this phenomenon is rare and should, therefore, be handled as exception to the rule.

HIV infection in children

Because newborns exposed to HIV by their infected mothers harbour HIV-specific antibodies, PCR-based tests are used in Switzerland for diagnosis of HIV infection. In contrast to vertically infected children, HIV diagnosis in previously HIV seronegative children can be made by serological test as for adults, and the same window period can be applied.

6. Current diagnostic window periods in recommendations of other countries

HIV testing has become a key intervention measure for reducing onward transmission as it reduces the number of people living with HIV, who are unaware of their infection, and therefore likely to be putting sexual partners at risk. Importantly, treatment guidelines now recommend that treatment is offered as soon as possible after HIV diagnosis (treatment as prevention). Accurate laboratory diagnosis of HIV is thus essential to identify those who could benefit from treatment, and to reassure persons who are not infected. Improvements in the analytical and diagnostic sensitivity of HIV screening tests during the early phase of HIV infection have led recently many European countries to change their HIV testing recommendations by reducing the follow-up period after a suspected HIV exposure.

Except for the harmonized general trend of reducing the follow-up period for 4th generation laboratory tests, guidelines still vary significantly between countries or organisations. Depending on type of HIV screening test and type of sample, the window period for detecting a new HIV infection ranges from 4

weeks (4th generation HIV blood laboratory tests) to 12 weeks (3rd generation HIV blood laboratory tests and rapid tests). Notably, the lower sensitivity of rapid tests during the early phase of HIV infection is differently dealt with in the different recommendations.

Table 4 Overview on selected recommendations in Europe

	HIV screening tests				References
	4th gen. laboratory test	**3rd gen. laboratory test**	**4th gen. rapid test**	**3rd gen. rapid test**	
UK	4 weeks	12 weeks	-	-	(24)
Sweden	6 weeks	6 weeks	8 weeks	8 weeks	(25)
France	6 weeks	-	-	12 weeks	(26)
Germany	6 weeks	12 weeks	12 weeks	12 weeks	(27)
EU	6 weeks	12 weeks	6 weeks	12 weeks	(28)

Some of the guidelines define for specific situations exceptions from these general rules:

- The Swedish Guidelines come up with different recommendations after infection with HIV-2: A follow-up period of 12 weeks is recommended after a possible exposure to HIV-2, since the presently used screening assays do not include HIV-2 p25 antigen detection, and since only limited information is available on the development of HIV antibodies during early HIV-2 infection.
- Follow-up after PEP or PrEP:
  It has been especially noted that the appearance of detectable virus and the development of antibodies may be delayed if HIV infection occurs despite and while on PEP or PrPE. Thus, the same prolonged follow-up period is recommended after PEP or PrEP as after exposure, but with counting starting after PEP or PrEP is completed.

7. Pros and Cons of the 12 week vs. 6/8 weeks rule

Recommendations have to balance public and personal interests. Below is a short list of reasons favouring or disfavouring a particular general window period:

General 12 weeks rule: Pro

One simple rule covers all situations (high uptake)
> 99% of all HIV infections detected, < 1% missed
Known since several years, wide acceptance of rule

General 12 weeks rule: Contra

Longer time of uncertainty
Inadequate waiting times for ≥95% of exposed persons
All tests valued identically, irrespective of test-related window period

General 6/8 weeks rule: Pro

Shorter period of uncertainty
Adequate waiting time for ≥95% of all exposed persons
Definition of best use for each test possible

General 6/8 weeks rule: Contra

Up to 5% of all new infections could be missed, which could jeopardize prevention measures (questionable)
Requires additional rules for specific situations (poorer uptake of rules)
Risk of missing more early HIV infections than with the 12 weeks rule

8. Concluding remarks

The simplicity of the 12 weeks rule made it very attractive, and easy to implement in the health care system or at voluntary counseling and testing sites. With the diagnostic tools available today and the informations gathered since the implementation of the 12 weeks rule it is evident that such a long window period is inappropriate for persons with a normally functioning immune system and that it should, therefore, no longer be maintained. The decision about the window period duration in a new recommendation will be influenced largely by the attitude towards maintaining or abandoning a safety margin. For the work group 2 a window period of six weeks is tenable, it is clear though, that without a specific recommendation to use PCR-based HIV testing during acute HIV infection, shortening the window period to 4 weeks will result in too many missed acute infections, and will therefore be counterproductive to the goal of ending HIV-transmission.

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