**Chapter 5**

***Molecular Testing for Human Immunodeficiency Virus***

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**Question 1**

**Place of molecular testing in the diagnosis of HIV-1 primary infection**

**Introduction** – Transmission of HIV-1 may occur via sexual exposure, by contaminated product of human origin or from infected mother to child. Primary infection with HIV-1 may be symptomatic or asymptomatic. In case of recent infection, the time at which people were exposed can often be estimated and it is very important to know precisely the chronology of HIV-1 contamination in order to anticipate the risk of transmission, notably in case of unprotected sexual exposure, in patients having received unsafe blood products or in healthcare workers exposed to the blood of an HIV-infected subject though needle stick injury.

**Primary question** – **In case of HIV-1 primary infection, what is the diagnostic marker that is the first to become positive in the peripheral blood of a subject recently exposed to that virus?**

1. HIV-1 RNA (quantified by NAT)
2. p24 antigen (tested by ELISA)
3. HIV-1 antibodies (tested by ELISA)
4. All of them at the same time

**Correct response** – A

**Explanation of Correct/Incorrect response** **–** As illustrated in Figure 1 of the corresponding chapter of the book (n°5), HIV-1 RNA is the first marker to become positive in a context of primary infection, approximately 10 days after the contaminating event. Very soon after, the p24 antigen test becomes positive in turn. By contrast, the HIV-1 antibodies, which result from the immune response of the subject, take longer time to appear; the mean duration of this “seroconversion window” is of 2 to 3 weeks, but it can be longer, notably in case of dual infection with another sexually-transmitted agent.

**First Reply Question** – The “seroconversion window” is particularly at risk of favoring the transmission of HIV-1 because the level of the viral load is usually very high during primary infection. In many developed countries including the USA, this risk is taken into consideration for the donation of blood by implementing a direct test for the diagnosis of HIV-1 infection. **What is the molecular test that is usually recommended for blood donors?**

1. Qualitative determination of plasmatic HIV RNA
2. Quantitative determination of plasmatic HIV RNA
3. Detection of cell-associated HIV DNA in blood
4. Combined test detecting several viral genomes including HIV-1 in blood

**Correct response** – D

**Explanation of Correct/Incorrect response** **–** All the cited tests are able to correctly identify the presence of HIV genomic material in the circulation of blood donors potentially at the stage of “seroconversion window” during HIV-1 primary infection. However, the combined tests detecting several viruses are usually required in order to detect hepatitis B and C virus genomes together with that of HIV-1.

**Second Reply Question** – The “eclipse phase” is defined as the period during which no viral marker is detectable after the viral contamination. **In the case of HIV-1 infection, what is the approximate duration of this “eclipse phase”?**

1. A few hours
2. Less than one week
3. About ten days
4. A few weeks

**Correct response** – c

**Explanation of Correct/Incorrect response** **–** As illustrated in Figure 1 of the corresponding chapter of the book (n°5), the mean duration of the eclipse period is of approximately ten days. During this phase, the viral load is low, which minimizes the risk of virus transmission.

**Question 2**

**Place of molecular testing in the diagnosis of HIV-1 infection**

**Introduction** – Since the beginning of the HIV-1 pandemic in the 1980s, almost 78 million people have been infected with the HIV-1 virus and about half of them died. Despite the absence of preventive vaccine and of definite cure, the availability of lifelong effective treatments with antiretroviral drugs has transformed HIV infection into a chronic disease. In the monitoring of the infectious process that is usually asymptomatic during the first years, the initial step consists in the identification of infected subjects. This diagnostic phase is crucial and obeys to well codified rules that must be perfectly understood.

**Primary question** - The diagnosis of HIV infection relies on the use of sophisticated serological tests combining the detection of antibodies to HIV-1 and HIV-2 and of the p24 antigen of HIV-1 in peripheral blood (antibody/antigen assay). In case of positive result, an HIV-1/HIV-2 differentiation immunoassay(also called “confirmatory assay”) must be implemented. **Why is nuclear acid testing (NAT) not systematically recommended for the screening of HIV infection?**

1. It is not sensitive enough
2. It is not cost-effective
3. The risk of false-positive result is too high
4. The result cannot be obtained within 24 hours

**Correct response** – B

**Explanation of Correct/Incorrect response** **–** NAT is extremely sensitive and false-positive results are very rare, except in case of amplicon contamination that can be prevented by the respect of quality rules and monitored by adequate controls. With most NAT assays, the results are available within a few hours. The main obstacle to the use of these tests for diagnosis purposes is their high cost that is not compatible with mass screening. Another limit of most NAT assays is the need for trained personnel for performing the tests.

**First Reply Question** – The Centers for Disease Control and Prevention (CDC) of Atlanta, USA, emitted in 2014 recommendations for the diagnosis of HIV-1 infection. According to these new rules, **under which circumstances the detection of plasmatic HIV-1 RNA is it recommended for the diagnosis of HIV-1 infection?**

1. In case of negative antibody/antigen screening test
2. In case of positive antibody/antigen screening test associated to the presence of HIV-2 antibodies and the absence of HIV-1 by HIV-1/HIV-2 differentiation immunoassay
3. In case of positive antibody/antigen screening test associated to the presence of HIV-1 antibodies and the absence of HIV-2 by HIV-1/HIV-2 differentiation immunoassay
4. In case of positive antibody/antigen screening test associated to the absence of reactivity to HIV-1 and HIV-2 by HIV-1/HIV-2 differentiation immunoassay

**Correct response** – D

**Explanation of Correct/Incorrect response** **–** The new algorithm proposed by the CDCs is reproduced in Figure 5.2 of the corresponding chapter of this book. According to this algorithm, NAT assays are recommended when a discrepancy is observed between a positive HIV screening test and the absence of reactivity by HIV-1/HIV-2 differentiation immunoassay. If HIV-1 NAT is negative, it must be concluded to a false-positive result of HIV screening and the subject can be declared as not infected by HIV-1. If HIV-1 NAT is positive, it must be concluded to a recent infection by HIV-1 (early primary infection) during the “seroconversion window” that corresponds to a transitory phase preceding the antibody response (see Figure 1 in chapter 5); the subject exhibiting such a profile must be tested again until the appearance of specific antibodies that confirm the recent HIV-1 infection.

**Second Reply Question** – In case of discrepancy between a positive screening test and a negative or undetermined HIV-1/HIV-2 differentiation immunoassay, what are the benefits of using a molecular assay for the detection of HIV-1 RNA?

1. It is cost-effective
2. It is able to clearly differentiate HIV-1 from HIV-2 infection
3. It is very sensitive
4. In case of negativity, it allows to identify false-positive results of HIV screening tests
5. All of these

**Correct responses** – E

**Explanation of Correct/Incorrect response** **–** This new strategy is cost-effective because the measure of the HIV-1 load is presently commonly performed in specialized laboratories and will be needed for the follow-up of the patient if the HIV-1 infection is confirmed. Cross-reactivity may occur in antibody responses to HIV-1 and HIV-2; the positivity of HIV-1 NAT will clearly confirm HIV-1 infection and exclude HIV-2 infection (dual infections with the two serotypes are exceptional). HIV-1 NAT is a very sensitive assay, even in newly-infected subjects lacking antibody response to HIV-1. The negativity of HIV-1 NAT allows concluding to a false-positive result of the HIV screening test, a situation that is not infrequent, even with the last generations of screening serological assays.

**Question 3**

**Place of molecular testing in the follow-up of HIV-1 infection**

**Introduction** – After a stage of primary infection that can be clinically symptomatic or asymptomatic, the subject infected with HIV-1 experiences a long phase of clinical latency during which the viral replication is ongoing in most cases, but at very variable levels. A minority of individuals, called long-term non progressors, stays asymptomatic for years without developing immunodeficiency. By contrast, most of the infected subjects, if not diagnosed and treated, develop in less than ten years a progressive loss of their immune functions, affecting principally the T repertoire; aids is characterized by an acquired immunodeficiency that results in the occurrence of opportunistic infections and/or cancers that are responsible for the death of patients. Consequently, once a subject is detected “seropositive” for HIV, whatever the stage of HIV infection, it is crucial to initiate a strict follow-up aimed at preserving or restoring the immune capital of the infected individual. This close surveillance relies on two kinds of biological markers: the determination of CD4+ T lymphocyte cell count, usually by using flow cytometry analysis, and the measure of the HIV-1 load.

**Primary question** – **What are the aims of the follow-up of HIV-1 infected subjects combining the determination of CD4+ T lymphocyte cell count and the measure of the HIV-1 load?** Three of the four following proposals are true. Which are they?

1. Early initiation of effective antiretroviral therapy (ART) in patients whose immune system does not control the viral replication
2. Identification of those untreated subjects who cured HIV infection spontaneously
3. Identification and follow-up of long-term non progressors
4. Detection of resistance to ART

**Correct responses** – A, C, D

**Explanation of Correct/Incorrect response** **–** The follow-up of HIV-1 infected subjects has different objectives according to the stage of HIV infection. At the time of the infection discovery (whatever the stage of infection), the aim of the follow-up is to evaluate the need of initiating an ART treatment. In case of low CD4+ T lymphocyte cell count or presence of HIV RNA in peripheral blood, a triple association of ART drugs is started (proposal a). In contrast, if the T cell response is conserved and that the HIV-1 load is undetectable, no treatment is required but the subject is regularly followed-up by repeating the same tests (proposal c). In treated patients, the follow-up of the viral load is also very useful to monitor the development of HIV resistance to ART (proposal d) that is suggested by an increase of HIV load in a treated patient. In contrast, proposal b is wrong: despite the fact that a very limited number of subjects, called post-treatment controllers, were shown to control HIV infection after a short initial phase of treatment, the spontaneous cure of untreated HIV-1 infection is exceptional if not impossible.

**First Reply Question** – **Which kind of HIV molecular test is recommended for the current measure of HIV-1 load?**

1. Qualitative determination of plasmatic HIV RNA
2. Quantitative determination of plasmatic HIV RNA
3. Qualitative determination of HIV DNA associated to circulating cells
4. Quantitative determination of HIV DNA associated to circulating cells

**Correct response** – B

**Explanation of Correct/Incorrect response** **–** The measure of a viral load implies that the test is quantitative, which excludes proposals a and c. The tests used on a routine basis for determining the viral load target circulating HIV RNA (proposal b). However, the parallel exploration of the DNA viral load has been proposed recently with the aim of quantifying the HIV reservoirs, both in blood (proposal d) and in different tissues, including notably the intestinal and the semen reservoirs (not proposed); consequently, proposal d can be accepted in combination to proposal b, but it is not routine practice.

**Second Reply Question** – In low-income areas, HIV-1 quantitative point-of-care (POC) molecular tests have been developed for the follow-up of HIV-infected subjects. They can be used as near-patient testing in different clinical settings when a rapid answer is required or when high-tech laboratory facilities are unavailable. They represent a good alternative to classical tests used for the quantification of plasmatic HIV RNA. **All these proposals concerning HIV-1 quantitative POC molecular tests are true but one. What are they?**

1. They are affordable
2. They do not need trained experimenters
3. They are more sensitive than classical quantitative NAT
4. They do not require sophisticated equipment

**Correct responses** – A, B, D

**Explanation of Correct/Incorrect response** **–** According to UNITAID, POC tests must be “ASSURED”, which means affordable (proposal a), sensitive, specific, user-friendly (proposal b), rapid and robust, equipment-free (proposal d), and deliverable to end users. In contrast, they are not more sensitive than classical quantitative NAT tests that represent the gold standard in terms of sensitivity and specificity (proposal c).

**Question 4**

**Place of molecular testing in the diagnosis of neonatal HIV-1 infection**

**Introduction** – In the absence of any intervention, the combined risk of mother-to-child (MTC) transmission of HIV-1 in utero and intra-partum is 15-30 percent; breastfeeding increases the risk to 20-45 percent. Factors of risk are age, maternal viral load, clinical stage of HIV-1 infection and presence or absence of therapeutic and/or prophylactic antiretroviral therapy (ART) in mother and infant. Since infected children have a high morbidity and mortality in the first 2 years of life, an early diagnosis is essential for establishing the infectious status of the neonate and, in case of infection, for initiating appropriate ART. This objective implies the need of sensitive tests for the diagnosis of HIV infection in newborns.

**Primary question** – **For which reason are serological tests not adapted to the diagnosis of HIV-1 contamination of neonates at birth?**

1. Presence of passive maternal antibodies
2. Risk of false-positive results due to cross-reactivity with endogenous retroviruses
3. Lack of sensitivity due to the small amount of anti-HIV antibodies
4. Immaturity of the immune system that is unable to produce specific antibodies during the first months of life

**Correct response** – A

**Explanation of Correct/Incorrect response** **–** The correct response is of course proposal a. Maternal antibodies of the IgG class have the property to cross selectively the placental barrier during pregnancy. At birth, distinguishing antibodies produced by the mother and the baby is not possible by using a serological test. The clearance of maternal IgG may last for up to 18 months. During this period, specific antibodies are of no use for the diagnosis of HIV infection in the child. In contrast, the other proposals are erroneous: there is no serological cross-reactivity between HIV-1 and endogenous retroviruses (proposal b); if infected, the neonate is able to produce large amount of specific antibodies (proposals c and d) but these antibodies are mixed to those of maternal origin.

**First Reply Question** – Molecular tests are imperatively needed for determining whether a neonate is infected or not by HIV-1 at birth. **Which of these NAT assays is not adapted for the diagnosis of HIV-1 contamination in neonates?**

1. Qualitative determination of plasmatic HIV-1 RNA
2. Quantitative determination of plasmatic HIV-1 RNA
3. Detection of HIV-1 DNA associated to circulating cells
4. Detection of HIV -1 RNA in whole saliva

**Correct response** – D

**Explanation of Correct/Incorrect response** **–** The aim of this diagnosis is to detect the presence of HIV-1 genome in blood with a sensitive and specific method. The tests cited in proposals a to c resume these characteristics. A quantitative test is not necessary but is adapted if it is as sensitive as a qualitative one. Usually, the plasmatic RNA is first performed; if positive, the diagnosis is confirmed by testing the presence of DNA associated to circulating cells. Although noninvasive, whole saliva is not a good medium for testing HIV-1 although this test could be positive in case of MTC HIV transmission. In contrast, saliva is currently used for detecting rubella virus or cytomegalovirus in newborns suspected of congenital infection by these agents.

**Second Reply Question** – **What are the World Health Organization’s recommendations for excluding HIV-1 contamination in a child born to infected mother?**

1. HIV screening at birth and 2 months after breastfeeding cessation
2. HIV screening at birth and 6 months after breastfeeding cessation
3. HIV screening at 4-6 weeks of life and 2 months after breastfeeding cessation
4. HIV screening at 4-6 weeks of life and 6 months after breastfeeding cessation

**Correct response** – C

**Explanation of Correct/Incorrect response** **–** The WHO recommends a systematic HIV-1 screening in all exposed newborns at 4-6 weeks of age by using DNA or RNA molecular test, both approaches having equal sensitivity rates. If negative, an additional testing must be performed 2 months after breast-feeding cessation. This strategy is aimed to contribute to the rise of an “HIV-free generation”, notably in those areas where the rate of HIV-infected pregnant women is very high.

**Question 5**

**Determination of the resistance of HIV-1 strains to antiretroviral drugs**

**Introduction** – Current antiretroviral therapy (ART) of HIV-1 infected individuals includes usually a combination of three drugs in order to avoid the emergence of resistant strains. More than 25 approved molecules belonging to different classes of antiretroviral are available, some of them being combined in the same pill for facilitating the daily observance of treatment. In most cases, this regimen is able to maintain the plasmatic viral load at an “undetectable” level and to preserve the immune defenses. In 2013, 12.9 million people living with HIV were receiving ART, of which 11.7 million from low- or middle-income countries. Drug resistance determination has become a key element for monitoring the efficacy of treatments and transmission of drug-resistant mutants.

**Primary question** – **Which of these viral targets can be screened in order to determine the resistance of HIV-1 strains to antiretroviral drugs?**

1. protease
2. reverse transcriptase
3. integrase
4. envelope glycoproteins
5. All of these

**Correct response** – E

**Explanation of Correct/Incorrect response** **–** These four genes can be screened for determining the efficacy of ART since they all code for targets of antiretroviral drugs. Protease inhibitors and nucleosidic/nucleotidic and non nucleosidic inhibitors of reverse transcriptase are the two main families of antiretroviral drugs. Integrase inhibitors and antagonists of virus entry targeting envelope glycoproteins were introduced more recently.

**First Reply Question** – **Which of these viral targets must be screened for determining the viral tropism of HIV-1 strains when entry inhibitors are used?**

1. protease
2. reverse transcriptase
3. integrase
4. envelope glycoproteins

**Correct response** – D

**Explanation of Correct/Incorrect response** **–** The viral tropism of HIV-1 strains is conditioned by the ability of a viral strain to use either CXCR4 or CCR5 as a co-receptor of the CD4+ molecule for viral entry into the cell via the envelope glycoproteins. Some entry inhibitors such as maraviroc target specifically the CCR5 co-receptor. If this drug is intended to be used, it must be verified before that the causative strain actually uses the corresponding co-receptor.

**Second Reply Question** – The determination of HIV-1 strains to antiretroviral drugs relies either on phenotypic assays based on cell culture or on genotypic assays based on molecular testing (sequencing). **With regard to the determination of antiretroviral resistance, which of these tests is the more extensively used for the surveillance of HIV-infected patients treated by antiretroviral drugs?**

1. Actual phenotype resistance assay
2. Virtual phenotype resistance assay
3. First generation sequencing assay (Sanger sequencing)
4. Next generation sequencing (NGS)

**Correct response** – C

**Explanation of Correct/Incorrect response** **–** The most popular assays that are used for ART resistance determination are the genotypic tests based on the Sanger sequencing of the target genes of antiretroviral drugs, namely protease, reverse transcriptase, integrase and envelope glycoproteins; the presence of given punctual mutations on these genes have been shown to predict resistance to the corresponding drugs (proposal c). The rapid development of NGS technology (proposal d) suggests that it will supplant Sanger sequencing in a near future. Phenotype resistance assays (proposals a and b) are fastidious, time-consuming and expensive; they remain limited to clinical trials involving new antiretroviral drugs.