BIOLOGICAL TESTING FOR HIV, HEPATITIS B AND C INFECTIONS

PROTO J.P. PLASSCHAERT S. SARTOR F. WALCKIERS D.

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LIST OF ABBREVIATIONS

CDC   center of disease control
DBS   dried blood spots
EIA   enzyme immunoassay
Elisa  enzyme-linked immunosorbent assay
HBV   hepatitis B virus
HBsAg hepatitis B surface antigen
anti-HBs antibody to HBsAg
anti-HBc antibody to HBcAg
IgM anti-HBc IgM class antibody to HBcAg (detected in acute infection)
IgG anti-HBc IgG class antibody to HBcAg (detected in chronic infection)
HBeAg hepatitis B e antigen
anti-HBe antibody to HBeAg
HBVDNA hepatitis B virus deoxyribonucleic acid
HCV   hepatitis C virus
anti-HCV antibody to HCV
HIV   human immunodeficiency virus
IDU   intravenous drug user
IFA   immuno fluorescent antibody assay
LIA   line immunoassay
PCR   polymerase chain reaction
RIBA  recombinant immunoblot assay
RIPA  radio-immuno precipitation assay
RT-PCR reverse transcription – polymerase chain reaction
TMA   transcription-mediated amplification
SUMMARY

This report aims to describe the different biological tests available for HIV, HBV and HCV infections and to provide elements to choose the more appropriate testing technique for epidemiological surveys, accounting for validity, feasibility and price. Advantages and disadvantages of different testing techniques are discussed. The choice of specimens collected depends on logistics, investigated populations and sites selected, and the testing strategy. Direct and indirect costs should also be considered in assessing the costs linked to the use of a testing technique.

1. Biological testing

HIV

The diagnosis of HIV infection is usually based on the detection of antibodies to HIV. Most people develop detectable antibodies within 3 months after infection, the average being 25 days; in rare cases, it can take up to 6 months.

In clinical settings, HIV is generally diagnosed using first an ELISA test, as screening test, and then a Western blot test, as confirmatory test if the ELISA test is found to be positive. Testing is generally carried out in accredited reference laboratories on blood samples collected by venous puncture. Based on literature review, the sensitivity and specificity of ELISA and Western blot tests exceed 99%. Rapid tests have also been developed to diagnose HIV infection: they allow laboratory analyses and medical counseling to be completed in a single visit, at least for negative cases. Because rapid tests are generally carried out in small laboratories, positive and equivocal results must be confirmed, requiring that a blood sample should be sent to an accredited reference laboratory, where it will undergo confirmatory testing. Licensed kits used for rapid HIV testing have the same sensitivity, specificity, and performance characteristics as screening methods currently used in reference laboratories. At least for now, rapid HIV screening tests will only be licensed for use by health-care professionals at the “point of care”. Advantages of rapid HIV screening tests include:

(i) improvement of clients’ satisfaction who can rapidly receive their results;
(ii) easier and safer to use,
(iii) enhancement of the autonomy of people to be tested since they will be able to choose between conventional testing and rapid testing,
(iv) more people would receive their results (only one medical counselling).

In the context of epidemiological surveys, blood sampling through venous puncture can be expensive and raises several practical problems, which have to be taken into consideration before implementing the survey. Blood sampling requires a trained medical staff as well as needles, syringes, and collection tubes. Safety issues must be considered. Compared to saliva, venous puncture increases the risk of infection for health-care workers and laboratory technicians through inadvertent contact with infected blood (higher concentration of antigens in blood, possible wounding due to the use of sharp collecting devices, and handling of tubes containing contaminated blood). However, blood sampling may reduce participation and raises concern about selection bias. In addition, venous puncture among injecting drugs users is often difficult because access to vein is poor. Blood samples also need appropriate handling in order to avoid haemolysis and bacterial contamination (maintenance of the cold chain is required if transfer to the laboratory exceeds 24 hours after the collection). However, the use of whole blood, serum, or plasma has two main advantages:

(i) a better screening of positive cases owing to the higher concentration of HIV antibodies in blood compared to oral fluids or urine, and
(ii) the possibility to carry out confirmatory testing and/or additional laboratory analyses with the same sample (HBV and HCV, e.g.).
In addition, more specialised laboratory analyses can eventually be considered (HIV typing, HIV sub-typing, antiretroviral resistance, e.g.). Blood sampling by venous puncture can be easily carried out when participating drug users are recruited in clinical settings.

Whole blood can also be collected by finger stick, a less invasive blood collection method. Dried blood spots (DBS) can then be collected on filter paper. DBS samples have the advantage of being easily transported, safer, and, at least, do not, temporally, require cold storage. Testing methods used for DBS samples appear to have a slightly lower sensibility and specificity (87-99%). Collection however requires some training.

Saliva which also contains HIV antibodies is an alternative to blood testing. However, concentration of HIV antibodies in saliva is lower compared to that found in blood. In numerous studies, several oral fluid tests have proved to be, in some cases, as valid as a standard EIA blood test. Saliva tests have been recommended and used for population surveys, surveillance programs, and personal screening. Advantages of HIV tests carried out on oral fluid samples include:

(i) ease of sampling collection (no need for medically trained staff) making it possible to collect samples in a variety of field settings, including non-clinical settings,
(ii) contact with contaminated sampled fluids is minimized,
(iii) greater acceptability for hard-to-reach populations compared to venous puncture.

Two main disadvantages should however be considered when using saliva as testing sample:

(i) confirmation of positive cases by testing saliva samples requires an additional blood sample, and
(ii) the concentration of antibodies in saliva samples may not be sufficient to detect truly positive cases.

In addition, the laboratory technique should be validated, i.e. the laboratory must ensure that the technique is able to reproduce previous published values on sensitivity and specificity. Currently, validated techniques are not widely spread. Quality control of the analytical methods can also be a problem since large volumes of saliva are much more difficult to obtain than blood. Saliva tests seem to be slightly less sensitive and specific than blood tests: based on a literature review, sensitivity and specificity of saliva tests range from 84 to 100%.

Currently, no truly "rapid" test using urine samples is available on the market, although technological advances may soon change this. Actually, urine EIA tests are less sensitive and less specific than blood-bases test, may require an additional specimen for confirmation and cannot be used to perform additional testing for special studies.

Three criteria should be considered for choosing an HIV testing strategy:

(i) objective of the testing : transfusion/transplant safety, epidemiological surveillance or HIV diagnosis,
(ii) sensitivity and specificity of the tests being used, and
(iii) HIV prevalence in the studied population.

Three strategies are recommended:

(i) one test for use in transfusion/transplant safety whatever the HIV prevalence rate, in HIV diagnosis in populations with HIV prevalence higher than 30% among persons with clinical signs or symptoms of HIV infection, and in epidemiological surveillance in populations with an HIV prevalence higher than 10%;
(ii) two tests for use in HIV diagnosis in populations with HIV prevalence lower than 30% among persons with clinical signs or higher than 10% among asymptomatic persons, and in epidemiological surveillance in populations with an HIV prevalence lower than 10%;
(iii) three tests for use in HIV diagnosis in populations with HIV prevalence lower than 10% among asymptomatic persons.
When HIV testing strategies involve more than one test, the selection of laboratory techniques in terms of sensitivity and specificity and the order in which they are used are important for obtaining valid results. First, the reagents used in the tests should contain different antigens. Second, the first test, also called the screening test, should be as sensitive as possible in order to detect all positive cases. The second test, called the confirmatory test, should be as specific as possible in order to ensure that all truly negative cases are identified. Testing on serum, plasma, or dried blood spots is therefore recommended since confirmatory testing can be carried out on a single sample, avoiding the collection of additional blood sample.

**HBV**

The diagnosis of HBV infection is generally made on the basis of serological markers detected by ELISA assay. Serologic markers of HBV infection vary depending on whether the infection is acute or chronic.

In acute HBV infections, hepatitis B surface antigen (HBsAg) is the first serologic marker to appear in blood. It can be detected as early as 1 or 2 weeks and as late as 11 or 12 weeks after infection by HBV. In persons who recover, HBsAg is no longer detectable in serum after an average period of about 3 months. Hepatitis B antigen (HBeAg) is generally detectable in patients with acute infection; its presence in serum correlates with higher concentrations of HBV and greater infectivity. Antibodies against HBeAg (Anti-HBe) becomes detectable during convalescence, after the disappearance of HBeAg, and remains detectable, generally 1 or 2 years after infection. Acute HBV infection can also be diagnosed on the basis of the detection of IgM class antibody to hepatitis B core antigen (IgM anti-HBc) in serum; IgM anti-HBc is generally detectable at the time of clinical onset and declines to sub-detectable levels within 6 months. IgG anti-HBc persist indefinitely as a marker of past infection. Anti-HBs, antibodies to hepatitis B surface antigen, become detectable during convalescence after the disappearance of HBsAg in patients who do not progress to chronic infection. The presence of anti-HBs following acute infection generally indicates recovery and immunity from re-infection.

In chronic HBV infection, HBsAg is detected in serum for at least 6 months and is associated to the absence of IgM anti-HBc. Both HBsAg and IgG anti-HBc remain persistently detectable, generally for life. HBeAg is variably present in these patients: level of viral activity or replication is assessed by testing for hepatitis B antigen (HBeAg) and hepatitis B DNA (HBV DNA) in the serum. In most cases, the chronic infection becomes "non-replicative" and the subjects lose serum HBeAg and develop antibodies against HBeAg. In some cases, "replicative" infection persists along with detectable serum HBeAg. In chronically infected individuals, infection can switch from "non-replicative" to "replicative" and vice-versa.

Biological tests routinely carried out include anti-HBc (blood and saliva), HBsAg (blood and dried blood spots), anti-HBs (blood) and IgM anti-HBc (blood).

Based on a literature review, the sensitivity and specificity of the different biomarkers of HBV are as follows:

- anti-HBc in saliva : 82 for sensitivity and more than 99% for specificity,
- HBsAg in DBS : 99%, both for sensitivity and specificity,
- HBsAg in blood : more than 99 % both,
- anti-HBc in blood : more than 80% for sensitivity and 90-99% for specificity,
- IgM anti-HBc in blood : 99 % both, and
- anti-HBs in blood : 99 % both.
HCV

Testing for the presence of antibodies to Hepatitis C virus (anti-HCV) is recommended for identifying persons with hepatitis C viral infection. Anti-HCV testing includes initial screening with an immunoassay. A negative screening result is interpreted as anti-HCV negative; typically, persons whose anti-HCV test results are negative are considered uninfected. A positive screening result should ideally be verified with an independent additional test having high specificity (RIBA or a nucleic acid test). Currently, the majority of laboratories report a positive result based on a positive screening test result only, and do not confirm these results with more specific serologic or nucleic acid testing. Multiple reasons exist regarding why laboratories do not perform additional testing for anti-HCV, including lack of an established laboratory standard for such testing, lack of understanding regarding the performance and interpretation of the screening and additional HCV test, and the high cost of the additional HCV test. In certain clinical settings, false positive anti-HCV results are rare because the majority of patients being tested have evidence of liver disease and the specificity of ELISA test is high (more than 99 %). The sensitivity of ELISA test is also very high: more than 95 %.

Recent studies using saliva and DBS for anti-HCV testing reported high sensitivity and specificity: for DBS, sensitivity ranging from 95 to 99 % and specificity from 99 to 100%; for saliva, sensitivity ranging from 85 to 99 % and specificity from 99 to 100%.

2. Selection of the biological sample to be tested according to the investigated infectious diseases

<table>
<thead>
<tr>
<th>Investigated infectious diseases</th>
<th>Number of test</th>
<th>Recommended biological sample *</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV</td>
<td>One test</td>
<td>DBS, Blood, saliva</td>
</tr>
<tr>
<td></td>
<td>Two tests</td>
<td>Blood, DBS</td>
</tr>
<tr>
<td>HBV</td>
<td>One test</td>
<td>Blood, DBS</td>
</tr>
<tr>
<td>HCV</td>
<td>One test</td>
<td>DBS, blood, saliva</td>
</tr>
<tr>
<td>HIV and HBV</td>
<td>HIV : one test</td>
<td>Blood, DBS, saliva</td>
</tr>
<tr>
<td></td>
<td>HIV : two tests</td>
<td>Blood, DBS</td>
</tr>
<tr>
<td>HIV and HCV</td>
<td>HIV : one test</td>
<td>DBS, blood, saliva</td>
</tr>
<tr>
<td></td>
<td>HIV : two tests</td>
<td>Blood, DBS</td>
</tr>
<tr>
<td>HBV and HCV</td>
<td>One test</td>
<td>Blood, DBS</td>
</tr>
<tr>
<td>HIV and HBV and HCV</td>
<td>HIV : one test</td>
<td>Blood, DBS, saliva</td>
</tr>
<tr>
<td></td>
<td>HIV : two tests</td>
<td>Blood, DBS</td>
</tr>
</tbody>
</table>

* in order of preference (accounting for sensitivity and specificity);
1 based on the prevalence of HIV in the population;
2 only HBsAg can be detected in DBS

Besides, nowadays in Europe, HIV, HBV and HCV tests have to meet quality criteria specified in the European Common Technical Specifications. These can be found at [http://www.pei.de/ivd/cts.htm](http://www.pei.de/ivd/cts.htm)
3. Costs consideration

Total cost \(^{(1)}\) of HIV, HBV, HCV testing, including 2 visits of GP \(^{(2)}\), Belgium, 2003

<table>
<thead>
<tr>
<th>Samples</th>
<th>Biological sample</th>
<th>Test</th>
<th>Price per analysis (€)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV (^{(3)})</td>
<td>Serum</td>
<td>Screening</td>
<td>70 – 75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Screening + confirmation</td>
<td>95 – 105</td>
</tr>
<tr>
<td></td>
<td>Saliva</td>
<td>Screening</td>
<td>70 – 75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Screening + confirmation (^{(4)})</td>
<td>95 – 105</td>
</tr>
<tr>
<td></td>
<td>Finger stick (rapid test)</td>
<td>Screening</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Screening + confirmation</td>
<td>90 - 95</td>
</tr>
<tr>
<td>HBV (^{(5)})</td>
<td>Serum</td>
<td></td>
<td>80 – 90</td>
</tr>
<tr>
<td></td>
<td>Saliva</td>
<td></td>
<td>75 – 90</td>
</tr>
<tr>
<td>HCV (^{(6)})</td>
<td>Serum</td>
<td>Screening</td>
<td>75 – 80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Screening + confirmation</td>
<td>120 – 140</td>
</tr>
<tr>
<td></td>
<td>Saliva</td>
<td>Screening</td>
<td>75 – 80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Screening + confirmation (^{(4)})</td>
<td>120 – 140</td>
</tr>
</tbody>
</table>

Notes : \(^{(1)}\) including direct and indirect costs; \(^{(2)}\) 2 x 30 €; \(^{(3)}\) on the basis of the detection of HIV antibodies; \(^{(4)}\) in serum; \(^{(5)}\) on the basis of the detection of hepatitis B markers (HBsAg, anti-HBc, anti-HBs); \(^{(6)}\) on the basis of the detection of HCV antibodies.
1. INTRODUCTION

The diagnosis of Human Immunodeficiency Virus (HIV), Hepatitis B (HBV) and Hepatitis C (HCV) infections is generally based on serological testing carried out on blood samples obtained by venous puncture. Nevertheless in epidemiological surveillance, where information is being collected to monitor the epidemic and to plan for needs, tests on specimens that can be collected with minimal training, under difficult field conditions, and with minimal risk of infection, would be required. Therefore saliva and dried blood spots are possible advantageous methods.

This report aims to describe the different biological tests available for HIV, HBV and HCV infections and to provide elements to choose the best testing technique for epidemiological surveys, accounting for validity, feasibility and price.

The validity of a test is a function of both its sensitivity and specificity. These indices are usually estimated by administering the test to a group of persons who have the disease and to another group who do not and then comparing the results. In fact, no test has a sensitivity of 100% and a specificity of 100%. Screening tests are designed to be highly sensitive to ensure that no positive person is missed. The price for this high sensitivity is a slightly reduced specificity: some persons who are negative will test false positive. Although sensitivity and specificity provide information about the validity of a test, they do not provide information about the predictive value of a positive or negative result. The predictive value of a result is the likelihood, expressed as a probability, that this result truly reflects the infectious status of a person, being either positive or negative. Sensitivity and specificity should theoretically be constant properties of a test, regardless of the population being tested. Predictive values, in contrast, vary even from one population to another because it is highly dependent on the prevalence of disease in the population being tested. For a test with a given sensitivity and specificity, the positive predictive value increases as the prevalence of the disease gets higher, whereas the inverse holds for the negative predictive value, i.e. it decreases as the prevalence of the disease increases.

In the case of drug users, the feasibility of using a test depends on the ease of sampling collection and its safety, and the acceptability of the sampling method for these hard-to-reach populations. The need to carry out confirmatory testing and/or additional laboratory analyses is another aspect to consider in choosing a biological sample to carry out the tests.

The price of the testing should take into consideration the direct and indirect costs (laboratory and medical resources).

The final decision whether or not to choose a test will depend on the severity of the disease and on the cost of further testing or treatment. The prevalence of the disease in the community, the cost of additional examinations that may be necessary, and the purpose for using the test must also be considered.
2. HIV TESTING

2.1. Introduction

Actually, HIV infection constitutes a public health problem because treated people still remain infected even if the clinical evolution of the disease can be slowed down, and owing to the endemic spread in some countries. HIV infection is contracted by non-protected sexual relation or by exposure to human fluids as blood. HIV can also be transmitted from mother to child through birth. Two major types of HIV have been identified so far: - HIV-1 is the most common epidemic type worldwide - HIV-2 is found mostly in Africa.

At least ten different sub-types of HIV-1 have also been identified.

The early detection of HIV infection is an important public health priority both for early treatment as well as for prevention of HIV transmission.

2.2. Screening and confirmatory tests

The diagnosis of HIV infection is usually made on the basis of the detection of HIV antibodies. Most people develop detectable antibodies within 3 months after infection, the average being 25 days; in rare cases, it can take up to 6 months. Serological tests for detecting HIV antibodies are generally classified as screening tests (sometimes referred to as initial tests) or confirmatory tests (sometimes referred to as additional tests). HIV tests contain HIV-1 and HIV-2 antigens and detect antibodies to both. Initial tests provide the presumptive identification of antibody-positive specimens, and additional tests are used to confirm whether specimens found reactive with a particular screening test contain specific HIV antibodies.

Alternative strategies using combinations of screening assays including rapid tests based on different antigen preparations and/or different test principles could provide reliable results.

The World health Organization (WHO) and UNAIDS recommended three criteria for choosing an HIV testing strategy: (i) objective of the test (transfusion/transplant safety, surveillance, diagnosis of HIV infection); (ii) sensitivity and specificity of the test(s) being used, and (iii) HIV prevalence in the population being tested. Regarding testing strategies, UNAIDS and WHO recommended the three following strategies for surveillance (Fig 1):

- **Strategy I** requiring one test for use in:
  - diagnosis in populations with an HIV prevalence > 30% among persons with clinical signs or symptoms of HIV infections
  - transfusion/transplant safety, for all prevalence rates
  - surveillance in populations with an HIV prevalence > 10% (unlinked anonymous testing for surveillance among pregnant women at antenatal clinics, e.g.).

- **Strategy II** requiring up to two tests for use in:
  - diagnosis in population with an HIV prevalence ≤ 30% among persons with clinical signs or symptoms of HIV infections or >10% among asymptomatic persons;
  - surveillance in population with an HIV prevalence ≤ 10% (e.g. unlinked anonymous testing surveillance among patients at antenatal clinics or sexually transmitted infections clinics).
• Strategy III requiring up to three tests for use in diagnosis in populations with an HIV prevalence ≤ 10% among asymptomatic persons.

STRATEGY I  
Transfusion/transplant safety, Surveillance

A1

A1+ Positive  A1- Negative

↓

A2

A1+; A2+ Positive  A1+; A2- Negative

STRATEGY II  
Surveillance, Diagnosis

A1

A1+ Positive  A1- Negative

↓

A2

A1+; A2+ Positive  A1+; A2- Negative

STRATEGY III  
Diagnosis

A1

A1+ Positive  A1- Negative

↓

A2

A1+; A2+ Positive  A1+; A2- Negative

A3

A1+; A2+; A3+ Positive  A1+; A2+; A3- Negative

A : assay (test); 1, 2, 3 : order of assays; + : reactive; – : non reactive

Figure 1: Schematic representation of the UNAIDS and WHO HIV Testing Strategies

For HIV testing strategies where more than one test may be required (strategies II and III), the selection of testing technologies and the order in which they are used are important for obtaining valid results. First, the tests should contain different antigens. Second, the sensitivity of the first test should be as high as possible, and the specificity of the second test should be as high as possible. The first test is the screening test, so it is necessary to use a highly sensitive test to detect all positives. Because a few false positives will occur, the second test (confirmatory test) needs to be highly specific to ensure that all truly negative test results are identified as negative. In addition, when strategy II or III is used, tests using serum, plasma, or dried blood spots are recommended so that multiple tests can be performed from a single specimen, avoiding collection of additional specimens.

Enzyme immunoassay (EIA) and enzyme-linked immunosorbent assay (ELISA) are the most commonly used tests for screening purposes (see appendix 2 for a detailed description of these tests). These tests can be performed with serum, plasma, urine, oral fluids, or dried blood spots. Sensibility and specificity of standard EIAs technologies using blood are high (> 99%). Figure 2 shows the algorithm for HIV testing and result reporting. If the screening test is non-reactive for HIV antibodies, the screening test is termed negative and no further testing is done. If reactive or inconclusive for HIV antibodies, a confirmatory test will be performed. Confirmatory tests are more specifically tuned to detect HIV antibodies than screening tests. The most commonly used confirmatory test is the Western blot. Others include: radio immuno precipitation assay (RIPA), immuno fluorescent antibody assay (IFA), line immunoassay (LIA), and polymerase chain reaction
(PCR) tests. If the confirmatory test is negative, the result of the screening test is considered to be a false positive, indicating the patient is not infected with HIV. If the confirmatory test is positive, a report is sent back with a “confirmed positive” statement. These patients should be given a follow up appointment for medical assessment and counselling. If the confirmatory test yields to an indeterminate result, the patient may have been recently infected with HIV and may be in process of seroconversion (sero-conversion almost always occurs within 3 months \(^{5,7,8}\)). An indeterminate result could also come from cross-reactive autoantibodies or to antibodies of other unrelated antigens. In this case, HIV testing should be repeated 1 or 2 months later to determine whether the confirmatory test will yield a positive result. If after this period the confirmatory test is still indeterminate, testing is considered negative.

![Figure 2: Laboratory algorithm for HIV testing](image)

**2.3. HIV diagnostic in clinical settings**

**2.3.1. Standard HIV testing**

Currently, the standard procedure for HIV testing involves sending blood samples to a central laboratory, where they are tested in batches (“batch testing”). Any blood sample being positive on the screening test (“ELISA” test,) undergoes a confirmatory test that is more attuned to detect specific HIV antibodies (generally, the “Western blot”) \(^{5,7}\). The results of the test are given to the health-care provider who ordered the test only after they have been confirmed. This means that the person getting tested must visit the health-care provider a second time to learn the result as well as for the counselling. This whole process can take one week (normally, Elisa positive results as well as indeterminate results must be confirmed by a Western blot test that normally takes more time compared to Elisa test).

All persons who are tested for HIV infection should ideally be counselled, both before the blood sample is collected and after the results are known. In addition, HIV screening must be accompanied by an inform consent of the person being tested \(^{5,7}\).

In Belgian clinical settings, the cost of such testing, is estimated to be 1-3 EUR for a negative test sequence and 20-25 EUR for a positive test sequence. However, considering both direct and
indirect cost (technology, human resources, and equipment), the actual cost may exceed 25 € per test.

2.3.2. Rapid testing

Rapid tests are those that can be done on-site where the blood sample is collected and yield a result within 30 minutes after the sample is taken. This means that the results can be provided to the person during a single visit to the testing site. Most of the research has focused on the use of these truly rapid tests, many of which generate results in 15 minutes or less. The test kits generally contain all reagents needed to run the assay: no additional reagents or equipment are required. Most HIV rapid tests contain HIV-1 and HIV-2 antigens and so detect both antibodies. When HIV antibodies are present in sufficient concentration in the sample, a colour reaction occurs along a test strip. Licensed rapid HIV test kits have the same sensitivity, specificity, and performance characteristics as screening methods currently used in accredited laboratories. This allows the health-care professional to complete the HIV testing and counselling at a single visit for those testing negative. However, false-positive results will occur, particularly among patients from populations with a low rate of HIV infection. This means that all positive results and all results that are equivocal must be confirmed, requiring that a blood sample should be sent to an accredited laboratory, where it will undergo confirmatory testing.

Rapid tests are useful for small laboratories, for laboratories without electricity or equipment, and for geographic areas with limited laboratory infrastructure.

At least for now, license will only be given to health-care professionals in order to use rapid HIV screening tests at the “point of care”. This distinguishes them from home test kits, which enable a person to collect the sample himself and either mail it to a laboratory and receive the test result by phone (home sample collection or home-access testing), or obtain the result within a few minutes (true home tests, also called home self-tests or home validated tests). Most rapid tests include an internal quality control. However, when using rapid tests, quality assurance and external quality control measures need to be developed and implemented at all sites that are using them.

Rapid tests can be performed with serum, plasma, and whole blood. With oral fluid, rapid test is under evaluation in field settings. No rapid test is currently available for use with urine.

Rapid tests generally cost between 2-4 €.

Potential benefits using rapid HIV screening tests at the point of care have been demonstrated:

- better satisfaction of tested persons because they can rapidly receive their results,
- easier and safer to use,
- people would be able to chose between conventional testing and rapid testing, enhancing their autonomy,
- more people would receive their test results, since most would not have to return for their results and post-test counselling,
- access to HIV screening could be improved, and
- acceptance of HIV testing could be increased.

In addition, it has been argued that rapid screening:

- is a mean to check the HIV status of pregnant women, if unknown at the time of labour. For those screening positive, to initiate preventive measures to reduce the risk of mother-to-child transmission,
- is useful to screen potential organ donors,
- could provide more information for decisions about post-exposure prophylaxis.

While there are potential advantages in using rapid HIV screening at the point of care, there are also some disadvantages:
- people undergoing rapid HIV screening will not receive adequate counselling (particularly people who receive a positive screening result, for whom provision of best-practice counselling and support is essential);
- rapid HIV screening test at the point of care must be accompanied by rapid access to confirmatory test, and support services must be easily accessible to people who receive a positive result of the screening tests.

2.4. HIV testing in epidemiological context

For diagnostic purposes, serologic evidence of HIV antibodies is currently the principal method of detecting infection. However, in epidemiological studies, specimens should be collected with minimal training and minimal risk of infection, and sometimes under difficult field conditions, Many types of specimens can be used with HIV testing technologies for HIV biological surveillance: whole blood, plasma, serum, oral fluids, and urine. The choice of specimens collected depends on logistics, populations and sites selected, and the HIV testing strategy. The specimens that cannot be tested on site will need to be transported to a laboratory for testing.

2.4.1. Whole blood, serum, and plasma

Testing for HIV antibodies is usually done on blood samples obtained by venous puncture. Collection of large numbers of blood samples for epidemiological studies may be expensive and difficult for many reasons; in the fieldwork, obtaining venous blood can be difficult and time consuming, especially in infants, small children, intravenous drug users. This may reduce participation and raises concerns about selection bias. Furthermore, venous puncture requires trained personnel as well as needles, syringes, and collection tubes. Safety issues must be considered. Compared to the sampling of saliva, venous puncture involves a higher risk of contamination for health-care workers and technicians through inadvertent wounding, both because of the use of sharp collecting devices as well as higher antibody concentration in sampled blood. When large numbers of samples are collected, field conditions may limit the prompt handling and refrigeration of the specimens, leading to haemolysis and possible bacterial contamination. Finally, transfer of the blood samples to the laboratory requires the maintenance of the cold chain (if transfer time exceeds 24 hours after the collection). However, whole blood, serum, and plasma have the following advantages:

- higher concentration of HIV antibodies than urine or oral fluids,
- possibility for confirmatory testing and additional routine testing (e.g., syphilis, Hepatitis B, Hepatitis C) from a single specimen,
- potential for special studies (e.g., HIV typing, HIV subtyping, antiretroviral resistance),
- easy to collect and test in clinical settings with a trained physician.

2.4.2. Dried blood spots

Needle stick methods have been used to draw small quantities of blood from finger or heel. Blood droplets are then collected on a filter paper card where they can dry (+ 2 hours at temperature room). Dried blood spots (DBS) have been used as a practical method of sample collection in serologic surveillance studies of numerous diseases, and for screening of diseases metabolism among neonates (“Guthrie card”). As described before, collection of blood samples by venous puncture presents a number of logistical challenges. The requirements of numerous supplies, staff trained in venous puncture, and refrigeration on the serum samples while they are being transferred to the central processing laboratory are difficult to meet and can be quite expensive. DBS are safe, lightweight, do not require, at least temporally, cold storage and have the advantage of being easily transported in a sealed plastic bag. However using DBS requires additional laboratory manipulation (dilution) which costs time and affects the accuracy of the test. Nevertheless DBS sensibility and specificity are quite high (87-99%). Collection requires some training.
2.4.3. Oral fluid

An alternative to blood testing is to use saliva which also contains HIV antibodies, but at lower concentration. In numerous studies, several oral fluid tests have proved to be as valid or, in some cases, as nearly valid as a standard EIA blood test. Saliva tests have been recommended and used for population surveys, surveillance programs, and personal-screening.

Several advantages of HIV tests using oral fluid samples have been described:
- easy collection of the biological sample,
- no need for medically trained staff for sample collection,
- elimination of the risk of needle-stick injuries,
- possibility to collect in a variety of field settings, including non-clinical settings.

Besides it has a greater acceptability to patients than drawing blood.

However, oral fluids have the following disadvantages:
- likely to cost more than using serum or plasma,
- may require an additional biological sample (blood) for confirmation,
- cannot be used to perform additional testing for special studies (HIV-1 vs HIV-2, HIV sub-typing, antiretroviral resistance, e.g.),
- possibility that saliva may not contain sufficient antibodies, and
- difficulties in obtaining large volumes of saliva for quality control.

Currently, rapid HIV test using oral fluids are under evaluation in the field settings. In 2000, researchers reported that the Saliva•Strip HIV™ rapid test developed by Saliva Diagnostic Systems had a specificity and positive predictive value of 100%; however, it was significantly less sensitive (94.6 %) and therefore had a lower negative predictive value (94.4 %) than existing blood-based EIAs. This means that while the test did not yield any false positive results, it did yield some false negative results. Researchers concluded that the sensitivity and negative predictive value were "adequate but not optimal.... For identification of all infected patients, a second assay with increased [sensitivity] is warranted." The manufacturer's earlier claim of a sensitivity of 99.4 % and specificity of 99.4 % do not correspond with the results obtained by these researchers.

The cost of saliva testing (only reactive costs) is similar than serum. However, saliva commercially test kits were developed. In Belgium the price per kit is about 126 € (Kit of 96 tests, Abbot Diagnostics). Cost linked to the use of saliva tests will be affected by the indirect costs. Normally, before implementing a saliva test in the study population, the central processing laboratory must validate and improve the replication of the test. This validation increases the cost of the HIV testing because serum samples have to be collected to confirm saliva test results.

In case that the screening test is positive, the person would be referred to a health care provider for counselling and diagnostic testing with blood.

2.4.4. Urine

The advantages of urine testing (as well as oral fluid) over blood testing include: easier use by health-care workers, eliminating accidental needle-sticks or other exposures to blood, being more acceptable to patients because blood needs not be drawn, less infrastructure required to collect samples and lower cost.

Currently, no truly "rapid" test using urine samples is available on the market, although technological advances may soon change this.

Actually, urine EIA tests are less sensitive and specific than blood-based tests, may require an additional blood sampling for confirmation and cannot be used to perform additional testing for special studies.
2.5. Sensitivity and specificity of HIV tests

Table 1: Sensitivity and specificity of saliva and blood tests for HIV testing (based on literature review)

<table>
<thead>
<tr>
<th>Biological sample</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saliva</td>
<td>82.5 - 100</td>
<td>81.8 - 100</td>
<td>1987 - 2002</td>
</tr>
<tr>
<td>Dried blood spots</td>
<td>87 - 100</td>
<td>99</td>
<td>1991 - 2002</td>
</tr>
</tbody>
</table>

1 A more detailed list of sensitivity/specificity figures published during the period 1987-1996 can be found at [http://www.ph.ucla.edu/epi/saliva.html](http://www.ph.ucla.edu/epi/saliva.html)

2.6. Comparison of HIV testing techniques

Advantages and limitations of standard HIV testing (EIA) and rapid testing are summarized in Table 2.
Table 2: Comparison of HIV testing techniques: Enzyme Immunoassays and Rapid Tests

<table>
<thead>
<tr>
<th>HIV testing Technique</th>
<th>Specimens</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EIA</strong> <em>(Standard HIV test)</em></td>
<td>Serum, Plasma, Whole blood, Dried blood spots, Oral fluids, Urine</td>
<td>Can be batched: good for ≥ 100 specimens at the time</td>
<td>Requires skilled, trained technicians to perform and read results.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quality assurance/ Quality control (QA/QC) done at national and regional laboratories: easier to control</td>
<td>Requires &gt; 2 hours for results (if need to run two EIAs, &gt; 5 hours)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cost per test less than cost per rapid test</td>
<td>Requires special equipment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Identifies sero-converters earlier: highly sensitive, which reduces non-reactive period</td>
<td>Requires maintenance of equipment</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Reagents must be refrigerated</td>
</tr>
<tr>
<td><strong>Rapid test</strong></td>
<td>Serum, Plasma, Whole blood, Oral fluids</td>
<td>Results in &lt; 30 minutes</td>
<td>Not good for testing &gt;100 specimens at a time</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Good for testing 1 to 100 specimens at a time</td>
<td>The QA/QC is performed at multiples sites: requires more control</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Requires minimal equipment and reagents</td>
<td>May cost more per individual test than EIA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Can be performed in a clinic (on-site testing)</td>
<td>All positive and equivocal results must be confirmed, requiring that a blood sample should be sent to an accredited laboratory</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Highly skilled staff not required</td>
<td>Inter-reader variability may provide inconsistent results with some assay format (particle agglutination, e.g.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Easy to interpret test results</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Test kits can be stored at room temperature</td>
<td></td>
</tr>
</tbody>
</table>

1. The cost linked to the use of a testing technique will be affected by the direct and indirect cost
2. Under evaluation in field settings
3. HEPATITIS B TESTING

3.1. Introduction

Hepatitis B virus (HBV) causes acute and chronic hepatitis. The chances of becoming chronically infected depend upon age. About 90% of infected neonates and 50% of infected young children will become chronically infected. In contrast, only 5% to 10% of acutely infected adults will develop chronic hepatitis B. In some individuals who become chronically infected, especially neonates and children, the acute infection will not be clinically apparent. Acute hepatitis B can range from subclinical disease to fulminant hepatic failure in about 2% of cases.

Actually, the major causes of HBV infection are non-protected sexual relations, use of unscreened blood transfusions, and re-use of needles and syringes that have not been adequately sterilized. Prevalence of hepatitis B infection among injecting drugs users range from 10 to 30%.

3.2. Diagnosis and screening

Some individuals with chronic hepatitis B will have no apparent clinical disorders of the liver or minimal liver disease and never develop complications. Others will have clinically apparent chronic hepatitis. Some will develop cirrhosis. Individuals with chronic hepatitis B, especially those with cirrhosis but even so-called chronic carriers, are at an increased risk of developing primary liver cancer.

The diagnosis of HBV infection is generally made on the basis of serology. Normally, serological markers of HBV infection are detected by ELISA assay.

Serologic markers of HBV infection vary depending on whether the infection is acute or chronic. The first serologic marker to appear following acute infection is the hepatitis B surface antigen (HBsAg), which can be detected as early as 1 or 2 weeks - and at the latest 11 or 12 weeks - after exposure to HBV (Figure 3). In persons who recover, HBsAg is no longer detectable in serum after an average period of about 3 months. The hepatitis Be antigen (HBeAg) is generally detectable in patients with acute infection; the presence of HBeAg in serum correlates with a bigger quantity of HBV and greater infectivity. The antibodies against HBeAg (Anti-HBe) become detectable during convalescence, after the disappearance of HBeAg, and remains detectable, generally 1 or 2 years after infection.

A diagnosis of acute HBV infection can be made on the basis of the detection of IgM class antibodies to hepatitis B core antigen (IgM anti-HBc) in serum; IgM anti-HBc is generally detectable at the time of clinical onset and declines to sub-detectable levels within 6 months. IgG anti-HBc persist indefinitely as a marker of past infection. Anti-HBs become detectable during convalescence after the disappearance of HBsAg in patients who do not progress to chronic infection. The presence of anti-HBs following acute infection generally indicates recovery and immunity from re-infection.
Chronic HBV infection is suggested by the presence of HBsAg in serum for at least 6 months and is confirmed by the absence of IgM anti-HBc (Figure 4). In patients with chronic HBV infection, both HBsAg and IgG anti-HBc remain persistently detectable, generally for life. HBeAg is variably present in these patients: level of viral activity or replication is assessed by testing for HBeAg and hepatitis B DNA (HBV DNA) in the serum.

Chronic infection with HBV can be either "replicative" or "non-replicative." In non-replicative infection, the rate of viral replication in the liver is low and serum HBV DNA concentration is generally low and HBeAg is not detected. In "replicative" infection, the patient usually has a relatively high serum concentration of viral DNA and detectable HBeAg. Patients with chronic hepatitis B and "replicative" infection defined by the presence of detectable HBeAg have a generally worse prognosis and a greater chance of developing cirrhosis and/or hepatocellular carcinoma than those without HBeAg.

In most cases, the chronic infection becomes "non-replicative" and the subjects lose serum HBeAg and develop antibodies against HBeAg. In some cases, "replicative" infection persists along with detectable serum HBeAg. In chronically infected individuals, infection can switch from "non-replicative" to "replicative" and vice-versa. One goal of treatment is to convert patients with chronic hepatitis B from a "replicative" (HBeAg positive) to a "non-replicative" (HBeAg negative) state.
The tables 3 and 4 summarize the different serological markers of hepatitis B in the different phases of infection.

**Table 3: Serological markers of hepatitis B in the different phases of infection**

<table>
<thead>
<tr>
<th>Serological marker</th>
<th>Acute infection</th>
<th>Chronic infection</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbsAg</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Anti-HBc : IgM</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anti-HBc : IgG</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anti-HBs</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
</tr>
<tr>
<td>HBV DNA**</td>
<td>+</td>
<td>+/-</td>
<td>-</td>
</tr>
<tr>
<td>HBeAg**</td>
<td>+</td>
<td>+/-</td>
<td>-</td>
</tr>
<tr>
<td>Anti-HBe**</td>
<td>-</td>
<td>+/-</td>
<td>+</td>
</tr>
</tbody>
</table>

+ : positive findings; - : negative findings; +/- : inconclusive findings
* Confers immunity. Hepatitis B immunization results only from anti-HBs production
** Usually ordered in chronic infections only

**Table 4: Interpretation of results of hepatitis B testing**

<table>
<thead>
<tr>
<th>Tests</th>
<th>Results</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg, anti-HBc, anti-HBs</td>
<td>negative</td>
<td>Susceptible</td>
</tr>
<tr>
<td>HBsAg, anti-HBc, anti-HBs</td>
<td>negative</td>
<td>Immune due to natural infection</td>
</tr>
<tr>
<td>HBsAg, anti-HBc, anti-HBs</td>
<td>positive</td>
<td>Immune due to hepatitis B vaccination</td>
</tr>
<tr>
<td>HBsAg, anti-HBc, IgM anti-HBc, anti-HBs</td>
<td>positive</td>
<td>Acute infection</td>
</tr>
<tr>
<td>HBsAg, anti-HBc, IgM anti-HBc, anti-HBs</td>
<td>negative</td>
<td>Chronically infected</td>
</tr>
<tr>
<td>HBsAg, anti-HBc, anti-HBc</td>
<td>positive</td>
<td>Possible interpretations:</td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>a. recovering from acute HBV infection</td>
</tr>
<tr>
<td></td>
<td>positive</td>
<td>b. distantly immune and test not sensitive enough to detect very low level of anti-HBs in serum</td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>c. susceptible with a false positive anti-HBc</td>
</tr>
<tr>
<td></td>
<td>positive</td>
<td>d. undetectable level of HBsAg present in the serum; the person is actually a carrier</td>
</tr>
</tbody>
</table>

Sensitivity and specificity of Anti-HBc diagnostic test are respectively 82% and 90–99%. Sensitivity and specificity of HBsAg, IgM anti-HBc and Anti-HBs are in the range 99-100%.

The cost of these HBV tests (only reactive price) is estimated to range from 1 to 3 €.
3.3. Methods for testing samples other than serum

Studies using saliva for anti-HBV testing were reported. Different markers can be tested in saliva: HBsAg, Anti-HBsAg, Anti-HBc. Sensitivity and specificity of HBV markers are respectively 82% and 99% for saliva (Anti-HBc) and both 99% for dried blood spots (HBsAg).

The costs of HBV tests (only reactive price) are similar than with serum. Cost of saliva test will be affected by the indirect costs. However, before implementing saliva test in the study population, central processing laboratory must validate and improve the replication of the test. This validation increases the cost of the HBV testing because serum samples have to be collected to confirm saliva test results.

3.4 Sensitivity and specificity of the different biomarkers of HBV

Biological tests routinely carried out include anti-HBc (blood and saliva), HBsAg (blood and dried blood spots), anti-HBs (blood) and IgM anti-HBc (blood).

Based on a literature review, the sensitivity and specificity of the different biomarkers of HBV are as follows:
- anti-HBc in saliva: 82% for sensitivity and more than 99% for specificity,
- HBsAg in DBS: 99%, both for sensitivity and specificity,
- HBsAg in blood: more than 99% both,
- anti-HBc in blood: more than 80% for sensitivity and 90-99% for specificity,
- IgM anti-HBc in blood: 99% both, and
- anti-HBs in blood: 99% both.
4. HEPATITIS C TESTING

4.1. Introduction

Hepatitis C (HCV) is a viral infection of the liver, which had been referred to as parenterally transmitted "non A, non-B hepatitis" until identification of the causative agent, in 1989.[24,57] HCV is a major cause of acute hepatitis and chronic liver disease, including cirrhosis and liver cancer. World-wide, unscreened blood transfusions and re-use of needles and syringes that have not been adequately sterilized are the major causes of HCV infection.[24,25,26,27,28] Studies on HCV risk factors indicate that the vast majority of prevalent HCV infections are through injecting drug use.[24,31,57,58] In injecting drugs users (IDUs), the prevalence rate of HCV ranged from 50 to 80%.[25,26,27,29,30]

4.2. Screening and confirmatory tests

Testing for the presence of HCV antibodies is recommended for initially identifying persons with hepatitis C virus infection. Testing for HCV antibodies should include use of an antibody-screening assay and, for positive results of this screening test, a more specific additional assay.[32]

Currently, the second-generation enzyme immunoassay (EIA-2.0) for HCV antibodies and HCV Version 3.0 ELISA are the most practical screening tests for detecting HCV infection. For EIAs, reactive specimens are retested in duplicate. All of these immunoassays use HCV-encoded recombinant antigens.[32,58,59] The sensitivity and specificity of EIAs are higher than 99 and 96%, respectively. The diagnosis of HCV infection can be supported or confirmed by the recombinant immuno-blot assay (RIBA), line immunoassays (LIA) or tests for HCV RNA.

HCV antibodies testing includes an initial screening with an immunoassay. A negative result is interpreted as anti-HCV negative; typically, persons whose anti-HCV test results are negative are considered uninfected. A positive result should be verified with an independent additional test having high specificity (RIBA or a nucleic acid test, Figure 3).

Currently, the majority of laboratories report a positive result based only on a positive result to screening test, and do not verify these results with more specific serologic assays or nucleic acid testing. Multiple reasons exist regarding why laboratories do not perform additional testing for anti-HCV, including lack of an established laboratory standard for such testing, lack of understanding regarding the performance and interpretation of the screening and additional HCV test, and the high cost of the additional HCV test. In certain clinical settings, false positive results in HCV antibodies testing are rare because the majority of patients being tested have evidence of liver disease and the specificity of Elisa tests is high (>99%). To promote the use of additional testing, the Centers for Diseases Control (CDC) have recommended to use the signal-to-cut-off ratio of screening test positive result to minimize the number of samples requiring an additional testing and provide result that has a high probability of reflecting the person's true antibody status. Screening test positive average s/co ratios $\geq 3.8$ would be highly predictive of RIBA positivity ($\geq 95\%$), with limited variability (95%-97%) and also were highly predictive of HCV RNA positivity, although the proportion that are HCV RNA positive is slightly lower than those for RIBA.[32]

The recombinant immunoblot assay (RIBA) is performed on positive results of the screening test. A negative RIBA result is interpreted as negative HCV antibodies and indicates a false positive result of the screening test. In this situation persons are considered uninfected. A positive RIBA result is interpreted as anti-HCV positive. Although the presence of anti-HCV does not distinguish between current or past infection, a confirmed anti-HCV positive result indicates the need for counseling and medical evaluation for HCV infection, including additional testing for the presence of virus (nucleic
acid test-NAT or HCV RNA) and liver disease (alanine amino-transferase, e.g.). Anti-HCV testing usually does not need to be repeated after the anti-HCV positive result has been confirmed. An indeterminate RIBA result can occasionally occur among recently infected persons in the process of serologic conversion, and among persons chronically infected with HCV. In this case, another sample should be collected for repeat anti-HCV testing (1 month later) or for HCV RNA testing (Figure 5). However, a high proportion of indeterminate RIBA results is usually attributable to false reactions.

**Figure 5:** Laboratory algorithm for antibody to hepatitis C virus (anti-HCV) testing and result reporting recommended by CDC.

Screening-test-positive results are classified as having high signal to cut-off ratios if their ratios are at or above a predetermined value that predicts an additional-test-positive result more than 95% of the time among all populations tested; screening-test-positive results are classified as having a low signal to cut-off ratio if their ratios are below this value.

---

ELISA-3.0 or EIA-2.0

---

HCV negative

---

POSITIVE

---

Epidemiological studies

---

Clinical purposes

---

HIGH SIGNAL TO CUTOFF RATIO

---

LOW SIGNAL TO CUTOFF RATIO

---

RIBA (Recombinant Immuno-Blot Assay)

---

HCV positive

---

Indeterminate

---

HCV negative

---

NAT (Nucleic Acid Test)

---

Negative

---

Positive

---

Figure 5: Laboratory algorithm for antibody to hepatitis C virus (anti-HCV) testing and result reporting recommended by CDC.

Screening-test-positive results are classified as having high signal to cut-off ratios if their ratios are at or above a predetermined value that predicts an additional-test-positive result more than 95% of the time among all populations tested; screening-test-positive results are classified as having a low signal to cut-off ratio if their ratios are below this value.
The nucleic acid tests (NAT) are commonly used in clinical practice as additional tests for the diagnosis of acute and chronic HCV infection and for evaluating and managing patients with chronic hepatitis C. The techniques used for HCV RNA detection \(^{32}\) are qualitative reverse transcription-polymerase chain reaction (RT-PCR) and transcription-mediated amplification (TMA).

If the NAT result is positive in persons with a positive result to the screening test, it has the advantage of detecting the presence of an active HCV infection as well as verifying the presence of HCV antibodies (Figure 3). If the NAT result is negative in persons with a positive result to the screening test, the HCV antibodies or infection status cannot be determined. Among persons with these results, additional testing with RIBA is necessary to verify the anti-HCV result and determine the need for counselling and medical evaluation. If the results of the anti-HCV screening test are judged falsely positive (i.e. RIBA-negative), no further evaluation of the person is needed; whereas if the results of the anti-HCV screening test are verified as positive by RIBA, the person should undergo medical evaluation.

Certain situations exist in which the HCV RNA result can be negative in persons with active HCV infection. HCV RNA is not detectable in certain persons during the acute phase of their hepatitis C, but this finding can be transient and chronic infection can develop \(^{32,34}\). In addition, intermittent HCV RNA positivity has been observed among persons with chronic HCV infection \(^{32,33}\).

In Belgium, the cost of HCV tests (only reactive price) were estimated \(^{32}\): 5 €/sample for initial screening test, 15 €/sample for those testing initially reactive and repeated in duplicate, 35–50 €/sample tested with RIBA; and 50–200 €/sample tested with a NAT.

The cost related to the use of a testing technique will be affected by the direct and indirect costs.

### 4.3. Methods for testing samples other than serum

High prevalences of HCV infection have been found among intravenous drug users. Blood sampling among injecting drugs users (IDUs) is often difficult because venous access is poor, and it is not always practicable for surveillance or screening programmes or in surveys where representative samples of the study population are sought. Methods for testing samples other than blood would be useful. Recently experiences using saliva \(^{35,36,37}\) and Dried Blood Spots \(^{38,39}\) for anti-HCV testing were reported. Saliva and DBS sensitivity and specificity are high (table 5).

<table>
<thead>
<tr>
<th>Biological sample</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried blood spots</td>
<td>95 - 99</td>
<td>99 - 100</td>
<td>1999 - 2000</td>
</tr>
</tbody>
</table>

In addition, many studies have shown an association between detectable salivary antibodies, high levels of serum antibodies and hepatitis C viraemia, suggesting that saliva could also be used as a surrogate marker for active hepatitis C infection \(^{37}\). Saliva test has also been used to examine the presence of HCV-RNA by RT-PCR \(^{40,41,42,43,44,45,49,50,51}\).

The costs of HCV saliva testing (only reactive costs) are similar to those of HCV testing in blood. Cost related to the use of a saliva test will be affected by indirect costs. However, before implementing saliva tests in the study population, central processing laboratory must validate and improve the replication of the test. This validation increases the cost of the HCV testing because serum samples have to be collected to confirm saliva test results.
5. CHOICE OF A BIOLOGICAL SAMPLE AND A TESTING METHOD: ELEMENTS TO BE CONSIDERED

5.1. Biological sampling: advantages and disadvantages

Table 6: Biological sampling

<table>
<thead>
<tr>
<th>Biological sampling</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLOOD</td>
<td>Higher concentration of serological markers than in urine or oral fluids</td>
<td>Require needles, tubes, or lancets, etc… which need to be destroyed in special treatment facilities for medical waste</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Higher risk for field workers through inadvertent wounding, both because of higher antigen concentrations and the use of sharp collecting devices</td>
</tr>
<tr>
<td>Blood collected by venous puncture</td>
<td>Possibility of additional testing from a single specimen</td>
<td>Require &quot;invasive&quot; collection technique with the difficulty to collect serum or plasma in non-clinical settings, and requiring skilled staff (for collecting and processing serum or plasma)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Require cold storage if transfer time to the laboratory exceeds 24 hours after the collection</td>
</tr>
<tr>
<td>Blood collected by finger stick (rapid test)</td>
<td>Less invasive blood collection method compared to venous puncture</td>
<td>No possibility of additional testing from a single specimen, a blood sample collected by venous-puncture is needed for confirmation</td>
</tr>
<tr>
<td></td>
<td>No laboratory transfer</td>
<td></td>
</tr>
<tr>
<td>Dried Blood Spot</td>
<td>Finger stick collection method</td>
<td>Calibration of the testing technique is required, i.e. the laboratory must ensure that the technique is able to replicate previous published values on sensitivity and specificity</td>
</tr>
<tr>
<td></td>
<td>Possibility of additional testing from a single specimen</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Samples easily transported without the need of a cold chain</td>
<td></td>
</tr>
<tr>
<td>ORAL FLUIDS, URINE</td>
<td>Ease of sampling collection (no need for medically trained staff) making it possible to collect samples in a variety of field settings, including non-clinical settings</td>
<td>No possibility of additional testing, a blood sample is needed for confirmation</td>
</tr>
<tr>
<td></td>
<td>Greater acceptability for hard-to-reach populations compared to the other specimen sampling</td>
<td>Calibration of the technique is required</td>
</tr>
<tr>
<td></td>
<td>Contact with contaminated sampling materials is minimized</td>
<td>Possibility that certain fluids may not have sufficient antibodies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Validated techniques for testing are not widely spread</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Urine EIA tests are less sensitive and specific than blood-based test</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No rapid test is currently available for urine samples</td>
</tr>
</tbody>
</table>
5.2. Selection of the biological sample to be tested according investigated infectious diseases

**Table 7: Selection of the biological sample**

<table>
<thead>
<tr>
<th>Investigated infectious diseases</th>
<th>Number of test</th>
<th>Recommended biological sample *</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV</td>
<td>One test</td>
<td>DBS, Blood, saliva</td>
</tr>
<tr>
<td></td>
<td>Two tests</td>
<td>Blood, DBS</td>
</tr>
<tr>
<td>HBV</td>
<td>One test</td>
<td>Blood, DBS</td>
</tr>
<tr>
<td>HCV</td>
<td>One test</td>
<td>DBS, blood, saliva</td>
</tr>
<tr>
<td>HIV and HBV</td>
<td>HIV : one test</td>
<td>Blood, DBS, 2, saliva</td>
</tr>
<tr>
<td></td>
<td>HIV : two tests</td>
<td>Blood, DBS</td>
</tr>
<tr>
<td>HIV and HCV</td>
<td>HIV : one test</td>
<td>DBS, blood, saliva</td>
</tr>
<tr>
<td></td>
<td>HIV : two tests</td>
<td>Blood, DBS</td>
</tr>
<tr>
<td>HBV and HCV</td>
<td>One test</td>
<td>Blood, DBS</td>
</tr>
<tr>
<td>HIV and HBV and HCV</td>
<td>HIV : one test</td>
<td>Blood, DBS, 2, saliva</td>
</tr>
<tr>
<td>HCV</td>
<td>HIV : two tests</td>
<td>Blood, DBS</td>
</tr>
</tbody>
</table>

* in order of preference (accounting for sensitivity and specificity);
1 based on the prevalence of HIV in the population;
2 only HBsAg can be detected in DBS

5.3. Total cost of HIV, HBV and HCV testing

**Table 8: Total cost *(1)* of HIV, HBV, HCV testing, including 2 visits of GP *(2)*, Belgium, 2003**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Biological sample</th>
<th>Test</th>
<th>Price per analysis (€)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV <em>(3)</em></td>
<td>Serum</td>
<td>Screening</td>
<td>70 – 75</td>
</tr>
<tr>
<td></td>
<td>Saliva</td>
<td>Screening</td>
<td>70 – 75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Screening + confirmation</td>
<td>95 – 105</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Screening + confirmation <em>(4)</em></td>
<td>95 – 105</td>
</tr>
<tr>
<td></td>
<td>Finger stick</td>
<td>Screening</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>(rapid test)</td>
<td>Screening + confirmation</td>
<td>90 - 95</td>
</tr>
<tr>
<td>HBV <em>(5)</em></td>
<td>Serum</td>
<td>80 – 90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Saliva</td>
<td>75 – 90</td>
<td></td>
</tr>
<tr>
<td>HCV <em>(6)</em></td>
<td>Serum</td>
<td>75 – 80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Saliva</td>
<td>75 – 80</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Screening + confirmation</td>
<td>120 – 140</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Screening + confirmation <em>(4)</em></td>
<td>120 – 140</td>
</tr>
</tbody>
</table>

*Notes: *(1)* including direct and indirect costs; *(2)* 2 x 30 €; *(3)* on the basis of the detection of HIV antibodies; *(4)* in serum; *(5)* on the basis of the detection of hepatitis B markers (HBsAg, Anti-HBc, Anti-HBs); *(6)* on the basis of the detection of HCV antibodies.
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47. Strip Immunoblot Assay (SIA) for the detection of antibodies to hepatitis C virus in serum or plasma: Hepatitis C Virus Encoded Antigen (Recombiant c-33c, c100 and c22 peptides). CHIRON RIBA HCV 3.0 SIA; 1999.


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58. Picazo, J. Diagnostico Serologico de la Hepatitis C. Protocolos de Diagnostico Serologico Clinico - Núm. 5.([www.fei.es/protocol/sero05.htm](www.fei.es/protocol/sero05.htm))

59. Lok, A. Diagnosis of Hepatitis C. Deutsches Hepatitis C Forum. Espenau, Germany ([www.hepatitis-c.de/diagnosti.htm](www.hepatitis-c.de/diagnosti.htm)).
APPENDIX 1: SENSITIVITY, SPECIFICITY AND PREDICTIVE VALUES OF A TEST

The validity of a test is a function of both its sensitivity and specificity. These indices are usually estimated by administering the test to a group of persons who have the disease and to another group who do not and then comparing the results. As shown in table 1, four categories of persons should be considered:
- “true positives” (TP), i.e. those who have the disease and have been tested positive;
- “false positives” (FP), i.e. those who actually do not have the disease but have been tested positive;
- “true negatives” (TN), i.e. those who do not have the disease and have been tested negative;
- “false negatives” (FN), i.e. those who do not have the disease and have been tested positive.

Table 1: Sensitivity, specificity of a test or diagnostic examination

<table>
<thead>
<tr>
<th>Test</th>
<th>Present</th>
<th>Absent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>TP</td>
<td>FP</td>
<td>TP + FP</td>
</tr>
<tr>
<td>Negative</td>
<td>FN</td>
<td>TN</td>
<td>FN + TN</td>
</tr>
<tr>
<td>Total</td>
<td>TP + FN</td>
<td>FP + TN</td>
<td>TP + FP + FN + TN = N</td>
</tr>
</tbody>
</table>

The sensitivity, $Se$, is the probability that the result of the test will be positive if the person is actually infected:

$$Se = \frac{TP}{TP + FN} = \frac{TP}{All\ people\ with\ the\ disease}$$

The specificity, $Sp$, is the probability that the result of the test will be negative if the person is not actually infected:

$$Sp = \frac{TN}{TN + FP} = \frac{TN}{All\ people\ without\ the\ disease}$$

In fact, no test has a sensitivity of 100% and a specificity of 100%. Screening tests are designed to be highly sensitive to ensure that no positive person is missed. The price for this high sensitivity is a slightly reduced specificity: some persons who are negative will test false positive.

Although sensitivity and specificity provide information about the validity of a test, they do not provide information about the predictive value of a positive or negative result. The predictive value of a result is the likelihood, expressed as a probability, that this result truly reflects the infectious status of a person, being either positive or negative.

The positive predictive value of a test, $PPV$, provides the probability that a person with a positive result is actually infected and can be obtain from:

$$PPV = \frac{TP}{TP + FP} = \frac{TP}{All\ people\ with\ a\ positive\ result}$$

The negative predictive value of a test, $NPV$, provides the probability that a person with a negative result is actually not infected and can be obtain from:

$$NPV = \frac{TN}{TN + FN} = \frac{TN}{All\ people\ with\ a\ negative\ result}$$
Sensitivity and specificity should theoretically be constant properties of a test, regardless of the population being tested. Predictive values, in contrast, vary even from one population to another because it is highly dependent on the prevalence of disease in the population being tested. Using the Bayes theorem, it can be demonstrated that:

\[ PPV = \frac{P \cdot Se}{P \cdot Se + (1-P) \cdot (1-Sp)} \]

\[ NPV = \frac{(1-P) \cdot Sp}{(1-P) \cdot Sp + P \cdot (1-Se)} \]

where \( P \) denotes the prevalence of the disease in the population.

For example, consider a test, which sensitivity is 97% and specificity is 99.8%. When the prevalence rate of the disease is 60%, the positive predictive value is 99.8% and the negative predictive value is 95.7%. Using the same test in a population where the prevalence of the disease is much lower, 0.3 cases per 1000, then the positive predictive value would be 12.7% and the negative predictive value would be 99.9%.

For a test with a given sensitivity and specificity, the positive predictive value increases as the prevalence of the disease gets higher, whereas the inverse holds for the negative predictive value, i.e. it decreases as the prevalence of the disease increases.

References

APPENDIX 2: PRINCIPLES OF HIV TESTING

1. Enzyme immunoassays

In any individual, antibodies are produced by the immune system as a protective response to the presence of invading foreign substances or organisms, including HIV antigens. These antibodies induce reactivity in any test designed to detect antibody-antigen reactivity, as do other non-specific factors, which may induce false reactivity, by acting like antibodies in the reaction. Most serological tests rely on the detection of these specific antibodies.

The principle of enzyme immunoassays (EIA) is based on the detection of antibodies after having form a complex antibody-antigen. The procedure includes the following steps:

1. the biological sample (blood, saliva, ...) is incubated with an antigen. This usually occurs in a well of microlitre plate, which is coated with antigen, though other solid phases such as glass beads may be used. Any specific or cross-reactive antibody present will bind to the antigen coating,
2. non-reactive antibodies in the biological sample are removed by washing,
3. a substance able to bind to antibody, the conjugate, is added to detect the antibody bound to the antigen. The conjugate itself carries a bound enzyme,
4. the excess conjugate is removed by washing,
5. a substance that reacts with the enzyme bound to the conjugate, the substrate, is added resulting in a colour change in those wells containing the conjugate (i.e. bound to antibody).

EIA’s for screening purposes recognise anti-HIV with high sensitivity and specificity (99%).

EIA technology has been adapted so that reaction can take place quite rapidly (30 minutes). The antigen is harnessed to membranes or similar supporting media and a single assay is carried out in a specially designed cartridge. The other reagents may be run across the membrane so that a colour reaction appears on the membrane. Thus reactivity may be detected rapidly in a single test format, with little or no laboratory equipment. It has been proposed that this type of test be used for rapid diagnosis under conditions other than normal diagnostic testing or screening conditions.

Characteristically, short incubation tests do not detect low affinity or low concentration antibody as well as the classic type of immunoassays, which employ longer incubation times allowing reactions to proceed to completion. It should be noted that rapid tests may identify anti-HIV negative samples reasonably well but, because of short incubation times, the assays do not always identify low affinity/low concentration antibody. Therefore, the potential for false negative results in short incubation tests is higher than that in EIAs using longer incubation periods and multiple antigens.

Rapid anti-HIV screening assays generally incorporate HIV-1 and HIV-2 recombinant or synthetic peptide antigens separately, allowing differentiation of HIV infection. No specific equipment is required for identification of positive reaction. However, these tests are expensive, only few tests can be done at a time and the end point is subjective. Stored contaminated or lipaemic samples can give erroneous results. Most of the rapid assays use only one synthetic peptide. Therefore early sero-converters or weak positive samples could be missed out.
2. Enzyme linked immunosorbent assays

Enzyme linked immunosorbent assays (ELISA) result from various modifications of EIAs concerning:

- **Solid phase**: micro-litre plate and polystyrene beads are the most commonly used solid phase in EIAs. Other solid phases can also be used: nitrocellulose membrane, nylon membrane, red blood cells, gelatine particles, latex particles, microscope slides, etc.

- **Source of antigenic material**:
  - Viral lysate is produced from T-cell culture material purified by density gradient ultracentrifugation. Antigen is separated by treatment with detergent and coated onto the solid phase. However, because of the preparation procedure, contaminating cellular antigens can decrease the specificity of the test by increasing false positive reactions.
  - Recombinant antigens are genetically engineered by inserting a portion of HIV genome in biological vectors such as E. coli. They generally contain limited number of HIV antigens. As a result only small amounts of non-viral antigens are available for false positive reactions.
  - Synthetic peptides are chemically synthesized amino acid residues corresponding to specific viral antigen epitopes. They are very pure and do not contain any contaminating cellular material. However, because of the relatively small antigens used they may not be able to detect HIV antibodies directed against conformational dependent epitopes present on larger antigen molecules.

- **Enzyme conjugate - substrate detection system**: alkaline phosphatases and horse radish peroxidase are the commonly used enzymes. While using alkaline phosphatases the most widely used substrate is para-nitrophenyl phosphate. Tetramethyl benzidine and orthophenylene diamine are also used as substrate in combination with horse radish peroxidase. A substrate is a reagent, which is degraded in the presence of the conjugate due to enzymatic activity. Horse radish peroxidase is generally preferred because of its low cost, easy conjugation to protein and wide variety of substrates that can be used in combination with it.

- **Sequence in which the reactants are used in the assay**: Depending on the sequence in which the reagents are added, the ELISAs can be grouped as follows:
  - **Indirect ELISA**: HIV antigens are coated onto the solid phase (wells of micro-litre plate or polystyrene beads). Antibodies if present in the sample will bind to the antigen, which can be subsequently detected using an enzyme labelled conjugate. A colour reaction is produced on addition of an appropriate substrate (figure 1). The intensity of the colouring is directly proportional to the antibody concentration present in the sera. This is the most commonly used ELISA type for antibody detection.
  - **Competitive ELISA**: In this assay the patient’s serum and enzyme labelled antibody (conjugate) are added simultaneously onto the solid phase (figure 2). If HIV antibodies are present in the patient’s serum they compete with the antibodies in the conjugate and reduce the binding capacity of the labelled antibody on the solid phase. The presence of colour in the reaction mixture on addition of substrate indicates absence of antibodies in the patient’s serum.
  - **Antigen sandwich ELISA** is a modified version of the indirect ELISA to increase the sensitivity and specificity. Components of this system are the same as in indirect ELISA except that an enzyme labelled antigen is used instead of enzyme conjugated antihuman immunoglobulin (figure 3). This assay detects all classes of HIV antibodies.
  - **Capture ELISA**: In the capture assay the solid phase is coated with an antibody agent (generally goat or sheep antihuman globulin), which captures antibody if present in the specimen being tested. Antibody is detected by using either Ag labelled with enzyme or antigen followed by an enzyme labelled antibody. The substrate added, changes colour in presence of the conjugate and is directly proportional to the amount of the antibody
Chemiluminescence: This assay is a modified ELISA involving use of light enhancing substances with the substrate. The advantage is that it is able to detect slight variation in optical density. However, it requires use of a special reader.

ELISA’s are easy to perform, can be automated for testing large number of samples. The reagents have relatively long shelf life of 6 to 12 months, they do not require use of radioisotopes and are sensitive and specific.

3. Particle agglutination assays

These assays are similar to EIAs except that the antigens are bound to an inert particle (i.e. gelatine particles). The particles act as the detection system because, in the presence of antibody, they agglutinate or assume visible dispersion patterns. These assays are highly reliable and have been used to great advantage in countries where there is limited funding for testing because they do not require sophisticated equipment.
4. Amplification assays (PCR).

Nucleic acid amplification methods for detection of viral genetic material have been developed. These tests include polymerase chain reaction (PCR) and branched DNA. They identify the presence of viral DNA or RNA by recognizing specific molecular sequences.

5. Western blots and line immunoassays.

The Western blot (WB) assay is a modified EIA where the individual HIV antigens are separated by electrophoresis and transblotted onto a solid support membrane, usually a nitrocellulose strip. Western blot for detection of HIV-1 and HIV-2 antibodies are available as kits where HIV-1 viral lysate antigens and HIV-2 specific envelope synthetic peptides are incorporated onto nitrocellulose strips. Detection kits for individual viral antibody are also available. A modification of the Western blot is the single strip line immunoassay (LIA), which uses synthetic peptides and recombinant antigens coated as discrete lines on a nylon strip with a plastic backing (figure 5). These are generally available as kits where major HIV-1 antigens representing the core proteins, envelope and the endonuclease domain of the viral polymerase gene and an antigen representing the transmembrane glycoprotein gp36 for the HIV-2 are incorporated. False positive and indeterminate results are minimised because these assays do not contain the contaminating cellular components.


Radio immunological-precipitation assay (RIPA) involves culturing HIV infected T lymphocytes in the presence of certain radio labelled amino acids. These are then lysed and the viral antigens are solubilised. Antibody, if present in the patient’s serum, is allowed to react with the viral lysate. The antigen-antibody complexes are precipitated by staph. protein A which binds to human immunoglobulin. Electrophoresis of this precipitate separates the labelled complexes by their molecular weight. The bands separated are visualised by autoradiography. This method is a research type assay, which can be used as an alternative to or in combination with WB. It is expensive, involves radioisotope handling and cultivation of HIV in culture.

Reference
## APPENDIX 3: HIV ANTIBODY TEST KIT – ANTIGEN AND TEST TYPES

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Test kit</th>
<th>Antigen type</th>
<th>Test type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbot</td>
<td>Recombinant HIV-1/HIV-2</td>
<td>Recombinant protein</td>
<td>Sandwich ELISA</td>
</tr>
<tr>
<td>Abbot</td>
<td>EIA, 3rd generation</td>
<td>Recombinant protein</td>
<td>Dot-immunoassay</td>
</tr>
<tr>
<td>Agen</td>
<td>SimpliRed</td>
<td>Synthetic peptide</td>
<td>Agglutination</td>
</tr>
<tr>
<td>Behring</td>
<td>Enzygnost HIV 1-2</td>
<td>Synthetic peptide</td>
<td>Indirect ELISA</td>
</tr>
<tr>
<td>Biotest Diagnostics</td>
<td>Biotest Anti HIV-1 HIV-2</td>
<td>Recombinant protein</td>
<td>Indirect ELISA</td>
</tr>
<tr>
<td>Cambridge Biotech</td>
<td>Recombigen HIV-1/ HIV-2</td>
<td>Recombinant protein</td>
<td>Agglutination</td>
</tr>
<tr>
<td>Du Pont de Nemours</td>
<td>Du Pont HIV-1/HIV-2 Elisa</td>
<td>Recombinant protein</td>
<td>Indirect ELISA</td>
</tr>
<tr>
<td>Fujirebio</td>
<td>Serodia-HIV</td>
<td>Lysate</td>
<td>Dot-immunoassay</td>
</tr>
<tr>
<td>Murex Diagnostics</td>
<td>Wellcozyme HIV Recombinant</td>
<td>Recombinant protein</td>
<td>Competitive ELISA</td>
</tr>
<tr>
<td>Murex Diagnostics</td>
<td>Wellcozyme HIV 1+2 Recombinant</td>
<td>Synthetic peptide / Recombinant protein</td>
<td>Sandwich ELISA</td>
</tr>
<tr>
<td>Organon Teknica</td>
<td>Vironostika Mixt</td>
<td>Lysate /Synthetic peptide</td>
<td>Indirect ELISA</td>
</tr>
<tr>
<td>Roche</td>
<td>Cobas Core</td>
<td>Recombinant protein</td>
<td>Indirect ELISA</td>
</tr>
<tr>
<td>Sanofi Diagnostic Pasteur</td>
<td>Genelavia Mixt</td>
<td>Synthetic peptide / Recombinant protein</td>
<td>Indirect ELISA</td>
</tr>
</tbody>
</table>

### Reference

APPENDIX 4: COLLECTING AND STORING BIOLOGICAL SAMPLES


Collecting Blood by Finger Stick

Blood collected by finger stick can be used to perform a rapid test or make a dried blood spot on filter paper. A dried blood spot may be preferred in rural settings and nonclinical settings, which often do not have trained staff and laboratory facilities with appropriate equipment (centrifuges, e.g.).

To obtain a finger-stick specimen,
1. massage the finger (preferably the middle or ring finger), which will cause blood to accumulate at the tip of the finger. The ear lobe may be pricked instead of the finger.
2. cleanse the finger pad (not just the tip or side of the finger) with 70% isopropyl (rubbing) alcohol. Wipe away alcohol with sterile gauze pad.
3. use a sterile lancet to firmly prick the finger pad. Wipe the first drop of blood off the finger with sterile gauze before collecting subsequent blood to place on the rapid test apparatus or on the filter paper for the dried blood spot. If the original puncture is inadequate, the same site should not be reused; another site or finger should be used.
4. avoid milking or squeezing the puncture as this may cause haemolysis of the blood and could invalidate the test result.

Preparing and Storing a Dried Blood Spot for an HIV Test

Blood from a finger or ear lobe stick can be used to make dried blood spots. Although finger stick is the most typical method, dried blood spots can also be obtained by using blood collected in a tube with an anticoagulant. Dried blood spots have the advantage of being easily transported, without the need for a cold chain.

1. Apply blood directly from a finger or a pipette onto special filter paper (Schleicher and Schuell Grade 903 Filter Paper or Whatman BFC 180 paper). The paper may come with preprinted circles that will contain approximately 100µL blood when completely filled. If the paper does not have preprinted circles, place blood on the paper so that it makes a circle with a 1.5 cm diameter. Allow the blood to soak through and fill the entire circle. Caution: If the blood does not saturate the filter paper, that paper should not be used.
2. Label the side of the filter paper with a code after the filter paper is saturated with blood (circle is filled).
3. Suspend filter paper strips containing the filled circles during the drying process to allow air to circulate around the paper. Stands for holding the strips are commercially available. However, strips may also be dried by placing them between two books (taping the edges of the strips to the books with sticky tape) on a table or a laboratory bench top so that the blood-containing part of the paper is not in contact with the surface of the table or laboratory bench top. Be sure not to get tape on the blood spot.
4. Let the blood spots air dry at room temperature for at least 4 hours (and for at least 24 hours in humid climates). Do not heat or stack blood spots, and do not allow them to touch other surfaces while they are drying.
5. After blood spots have been adequately dried, wrap the strip in one sheet of glassine paper or plastic to prevent carryover of specimen from one sheet to another.

6. Place the wrapped strips in a gas-impermeable bag with desiccant and humidity indicator cards. Approximately 20 strips may be placed in each bag. Bags may be kept at room temperature for up to 30 days and then stored at 4°C for up to 90 days. If the dried blood spots in their plastic bags are to be stored longer than 90 days, they should be maintained at -20°C. Properly stored dried blood spots have been shown to be stable for at least 2 years. The bags should be placed in a sturdy envelope for shipment.

Collecting and Storing Urine and Oral Fluids

Collecting Urine and Oral Fluids

A variety of EIA protocols exist for using urine specimens; however, no rapid tests are available at this time for use with urine specimens.

A few important considerations when collecting urine are:
1. a minimum of 200 µL of urine is usually required for use with a urine-based EIA test kit;
2. specimen can be collected at any time of day and need not be midstream;
3. a preservative may be added to the specimen for storage, although it is not required.

Oral Fluids

Oral fluids can be used with both modified EIAs and rapid tests. Rapid tests using oral fluids are currently under field evaluation. The following general steps to collect a specimen are provided:
1. use a specially treated absorbent pad attached to a plastic stick (usually provided by the test kit manufacturer);
2. place the pad in the person’s mouth against the inner cheek for the length of time specified in the manufacturer’s instructions. Then place the pad into a vial containing a preservative solution (usually provided by the test kit manufacturer). Due to the test complexity, oral fluid specimens collected for EIAs are sent to the central laboratory for analysis.

Storing Urine and Oral Fluids

Urine specimens should be placed in plastic cryo-vials containing a preservative. Urine specimens with a preservative may be stored up to 1 year at 4-8°C. Urine specimens for HIV testing must not be frozen.

Oral fluid specimens can be stored at 4-37°C for a maximum of 21 days (including the time for transfer and testing). Oral specimens should be refrigerated during transfer. Specimens can be frozen (-20°C) for a limited time (approximately 6 weeks). Once thawed, they can be refrozen once. Consult the test kit insert prior to testing for more specific storage information.
## APPENDIX 5: DETAILS OF COST EVALUATIONS

Table 1: Cost of HIV, HBV and HCV testing (only reactive price), Belgium, 2003

<table>
<thead>
<tr>
<th>Test type</th>
<th>Test Kit</th>
<th>Manufacturer</th>
<th>Price of kit (£)</th>
<th>Number of tests per kit</th>
<th>Price per test (£)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV: Elisa</td>
<td>Murex HIV-1.2.O</td>
<td>Abbot</td>
<td>126</td>
<td>96</td>
<td>1.3</td>
</tr>
<tr>
<td>HIV: Elisa</td>
<td>Murex HIV-1.2.O</td>
<td>Abbot</td>
<td>507</td>
<td>480</td>
<td>1.0</td>
</tr>
<tr>
<td>HIV: Elisa, finger stick (rapid test)</td>
<td>Determine HIV 1-2</td>
<td>Abbot</td>
<td>58</td>
<td>20</td>
<td>3.0</td>
</tr>
<tr>
<td>HIV: Western blot</td>
<td></td>
<td>Abbot</td>
<td>349</td>
<td>18</td>
<td>20.0</td>
</tr>
<tr>
<td>HIV: LIA</td>
<td>-</td>
<td>Immuno Lia confirmation</td>
<td>429</td>
<td>20</td>
<td>22.0</td>
</tr>
<tr>
<td>HBV: HBs-Ag</td>
<td>ETI-MAK 4</td>
<td>Diasorin</td>
<td>238</td>
<td>180</td>
<td>1.3</td>
</tr>
<tr>
<td>HBV: Anti-HBc</td>
<td>ETI-AB-COREK-2</td>
<td>Diasorin</td>
<td>267</td>
<td>90</td>
<td>3.0</td>
</tr>
<tr>
<td>HBV: IGM anti HBc</td>
<td>ETI-CORE-IGMK-2</td>
<td>Diasorin</td>
<td>274</td>
<td>96</td>
<td>3.0</td>
</tr>
<tr>
<td>HBV: Anti-HBs</td>
<td>ETI-AB-AUK-3</td>
<td>Diasorin</td>
<td>238</td>
<td>180</td>
<td>1.3</td>
</tr>
<tr>
<td>HCV: Elisa</td>
<td>Anti-HCV 3.0 ORTHO</td>
<td>Ortho Clinical Diagnostics (Diasorin)</td>
<td>1943</td>
<td>480</td>
<td>4.0</td>
</tr>
<tr>
<td>HCV: Elisa</td>
<td>Anti-HCV 3.0 ORTHO</td>
<td>Ortho Clinical Diagnostics (Diasorin)</td>
<td>1077</td>
<td>180</td>
<td>6.0</td>
</tr>
<tr>
<td>HCV: RIBA</td>
<td>CHIRON RIBA HCV 3.0 SIA</td>
<td>Ortho Clinical Diagnostics (Diasorin)</td>
<td>1487</td>
<td>30</td>
<td>50.0</td>
</tr>
<tr>
<td>HCV: LIA</td>
<td>Inno-Lia HCV AbIII</td>
<td>Innogenetics</td>
<td>515</td>
<td>20</td>
<td>25.7</td>
</tr>
</tbody>
</table>
### Table 2: The indirect costs of a serum testing technology, Belgium, 2003

<table>
<thead>
<tr>
<th>Resources</th>
<th>Type</th>
<th>Manufacturer</th>
<th>Price (€)/material</th>
<th>Price (€)/Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Material resources</strong></td>
<td>Transfer pipettes from LD-PE (1 ml)</td>
<td>MLS</td>
<td>12,88 €/ 20</td>
<td>0,02 €</td>
</tr>
<tr>
<td></td>
<td>Tubes 2 ml (12,5 X 49 mm)</td>
<td>MLS</td>
<td>212 €/1000</td>
<td>0,20 €</td>
</tr>
<tr>
<td></td>
<td>Cryoboxes tubes (136 x 75 ml)</td>
<td>MLS</td>
<td>121 €/20</td>
<td>0,10 €</td>
</tr>
<tr>
<td><strong>Laboratory resources</strong></td>
<td>Tips (1100pl)</td>
<td>Diasorin</td>
<td>577,10 €/10 plaques (1 plaque = 96 tips)</td>
<td>0,60 €/ tips</td>
</tr>
<tr>
<td></td>
<td>Tips (300 pl)</td>
<td>Diasorin</td>
<td>429,60 €/10 plaques (1 plaque = 96 tips)</td>
<td>0,44 €/ tips</td>
</tr>
<tr>
<td></td>
<td>Tips (micropipettes)</td>
<td>Merk Eurolab</td>
<td>29,10 €/1000</td>
<td>0,03 €</td>
</tr>
<tr>
<td><strong>S-Total</strong></td>
<td></td>
<td></td>
<td>1, 40 €</td>
<td></td>
</tr>
<tr>
<td><strong>Human resources</strong></td>
<td>Trained technician</td>
<td></td>
<td>0,30 €/ sample</td>
<td></td>
</tr>
<tr>
<td><strong>S-Total</strong></td>
<td></td>
<td></td>
<td>0.30 €</td>
<td></td>
</tr>
<tr>
<td><strong>Material resources</strong></td>
<td>Mailing container (115 x 29 mm)</td>
<td>MLS</td>
<td>176, 50 €/1000</td>
<td>0,20 €</td>
</tr>
<tr>
<td></td>
<td>Serum tubes with separation gel (9,5 ml) (100 x 16 mm)</td>
<td>Bechon Dickinson</td>
<td>582 €/100</td>
<td>5,8 €</td>
</tr>
<tr>
<td><strong>Medical resources</strong></td>
<td>Sample needle thin wall (25 mm)</td>
<td>Bechon Dickinson</td>
<td>8 €/100</td>
<td>0,10 €</td>
</tr>
<tr>
<td></td>
<td>Polyethen-envelopes (18 cm x 260 cm)</td>
<td>Bruneau</td>
<td>31 € /100</td>
<td>0,30 €</td>
</tr>
<tr>
<td></td>
<td>Label (52 x 21.2 mm)</td>
<td>Buropapier</td>
<td>9,75 €/1box (1 box = 5600 labels)</td>
<td>0,001 €</td>
</tr>
<tr>
<td></td>
<td>Label (63,5 x 33,9 mm)</td>
<td>Gilbert</td>
<td>38,99 €/1 box (1 box = 5600 labels)</td>
<td>0,007 €</td>
</tr>
<tr>
<td></td>
<td>Label (100 X 57 mm)</td>
<td>Gilbert</td>
<td>36,57 €/1 box (1 box = 5600 labels)</td>
<td>0,006 €</td>
</tr>
<tr>
<td></td>
<td>Post-pack boxes model D (l= 358 mm B= 215 mm H= 135 mm)</td>
<td>Post</td>
<td>300 €/ 20 (1 post-pack = samples)</td>
<td>2,5 €</td>
</tr>
<tr>
<td><strong>S-Total</strong></td>
<td></td>
<td></td>
<td>9 €</td>
<td></td>
</tr>
<tr>
<td><strong>Human resources</strong></td>
<td>General practitioner</td>
<td></td>
<td>30 € / visit (2 visits)</td>
<td></td>
</tr>
<tr>
<td><strong>S-Total</strong></td>
<td></td>
<td></td>
<td>60 €</td>
<td></td>
</tr>
<tr>
<td><strong>INDIRECT RESOURCES TOTAL</strong></td>
<td></td>
<td></td>
<td>71 €</td>
<td></td>
</tr>
</tbody>
</table>
Table 3: The indirect costs of a saliva testing technology, Belgium, 2003

<table>
<thead>
<tr>
<th>Resources</th>
<th>Type</th>
<th>Manufacturer</th>
<th>Price (€)/ material</th>
<th>Price/Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Material</strong></td>
<td><strong>resources</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transfer pipettes from LD-PE (1 ml)</td>
<td>MLS</td>
<td>12.88 €/ 20</td>
<td>0.02 €</td>
<td></td>
</tr>
<tr>
<td>Tubes 2 ml (12.5 X 49 mm)</td>
<td>MLS</td>
<td>212 €/ 1000</td>
<td>0.20 €</td>
<td></td>
</tr>
<tr>
<td>Cryoboxes tubes (136 x 75 ml)</td>
<td>MLS</td>
<td>121 €/ 20 (1 cryobobes tubes = 81 samples)</td>
<td>0.07 €</td>
<td></td>
</tr>
<tr>
<td>Tips (1100pl)</td>
<td>Diasorin</td>
<td>577.10 €/10 plaques (1 plaque = 96 tips)</td>
<td>0.60 €/ tips</td>
<td></td>
</tr>
<tr>
<td>Tips (300 pl)</td>
<td>Diasorin</td>
<td>429.6 €/10 plaques (1 plaque = 96 tips)</td>
<td>0.44 €/ tips</td>
<td></td>
</tr>
<tr>
<td><strong>Laboratory</strong></td>
<td><strong>resources</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tips (micropipettes)</td>
<td>Merk Eurolab</td>
<td>29.10 €/1000</td>
<td>0.03 €</td>
<td></td>
</tr>
<tr>
<td>Foetal calf/bovine serum (100 ml)</td>
<td>Invitrogen S.A.</td>
<td>23 €</td>
<td>0.02 €</td>
<td></td>
</tr>
<tr>
<td>Phosphate Buffer Saline</td>
<td>OXOID</td>
<td>20 €</td>
<td>0.002 €</td>
<td></td>
</tr>
<tr>
<td>Gentamicin (50 mg/ml) 20 ml</td>
<td>Invitrogen S.A.</td>
<td>28 €</td>
<td>0.007 €</td>
<td></td>
</tr>
<tr>
<td>Fungizone (250 µg/ml) 20 ml</td>
<td>Invitrogen S.A.</td>
<td>6.5 €</td>
<td>0.0003 €</td>
<td></td>
</tr>
<tr>
<td>Tween 20 polyoxyethylene orbitan (500 ml)</td>
<td>Sigma Aldrich</td>
<td>39 €</td>
<td>0.0001 €</td>
<td></td>
</tr>
<tr>
<td><strong>S-Total</strong></td>
<td>1.40 €</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Human</strong></td>
<td><strong>resources</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trained technician</td>
<td></td>
<td>0.30 €</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>S-Total</strong></td>
<td>0.30 €</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Material</strong></td>
<td><strong>resources</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oracol swabs</td>
<td>Malvern Medical Development</td>
<td>476 €/500</td>
<td>1 € (2 per sample)</td>
<td></td>
</tr>
<tr>
<td>Polyethen-envelopes (18 cm x 260 cm)</td>
<td>Bruneau</td>
<td>31 € /100</td>
<td>0.30 €</td>
<td></td>
</tr>
<tr>
<td>Label (52 x 21.2 mm)</td>
<td>Buropapier</td>
<td>9.75 €/1box (1 box = 5600 labels)</td>
<td>0.01 €</td>
<td></td>
</tr>
<tr>
<td><strong>Medical</strong></td>
<td><strong>resources</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Label (63.5 x 33.9 mm)</td>
<td>Gilbert</td>
<td>38.99 €/1 box (1 box = 5600 labels)</td>
<td>0.007 € (2 per sample)</td>
<td></td>
</tr>
<tr>
<td>Label (100 X 57 mm)</td>
<td>Gilbert</td>
<td>36.57 €/1 box (1 box = 5600 labels)</td>
<td>0.006 € (2 per sample)</td>
<td></td>
</tr>
<tr>
<td>Post-pack boxes model D</td>
<td>Post</td>
<td>300 €/ 20 (1 post-pack = 6 samples)</td>
<td>2.5€</td>
<td></td>
</tr>
<tr>
<td><strong>S-Total</strong></td>
<td>4.90 €</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Human</strong></td>
<td><strong>resources</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>General practitioner</td>
<td></td>
<td>30 € / visit (2 visit)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>S-Total</strong></td>
<td>60 €</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>INDIRECT</strong></td>
<td><strong>RESOURCES</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td>71.50 €</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>