

Laboratory methods for diagnostics of HIV infection and HIV-associated neuroinfections

Pavol BENO¹, Alzbeta KAIGLOVA¹, Martin SAMOBYL²

¹ Department of Laboratory Medicine, Faculty of Health Sciences and Social Work, Trnava University in Trnava, Slovak Republic

² Institute of Hygiene, Faculty of Medicine, Comenius University in Bratislava, Slovak Republic

Correspondence to: Assoc. Prof. Pavol Beno, PharmD., PhD.
Department of Laboratory Medicine in Health Care
Faculty of Health Sciences and Social Work, Trnava University
Univerzitne namestie 1, Trnava 918 43, Slovak Republic.
E-MAIL: pavol.beno@truni.sk

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Abstract

HIV Infection resulting in AIDS remains serious global public health problem. In the fight with the global health problem plays a key role a simple, reliable and fast diagnostics. An important method in diagnostics is the identification and detection of viral capsid p24 antigen levels. Fourth generation tests for the diagnostics of HIV infection simultaneously detect the presence of HIV antibodies and p24 antigen. Based on the monitoring of CD4 count, we can estimate the stage in which the infection is, and we can also suggest a therapeutic approach. Cerebral toxoplasmosis is the most common neurological opportunistic disease manifested in HIV infected patients. Cryptococcal meningitis is the second most common cause of the opportunistic neuroinfections. Despite of significant advances in the diagnostics and treatment of HIV infection, this disease is still unable to get completely under control. The future perspective in HIV diagnostics are biosensors.

INTRODUCTION

Worldwide, approximately 35 million people are infected with HIV and 2 million infections occurring each year; about 25 million of these live in sub-Saharan Africa. The HIV pandemic remains a significant public health problem (Okano *et al.* 2016; Cummins & Badley 2015). Slovak Republic still belongs to the countries with relatively low HIV epidemic in European Union. 577 cases of HIV was registered from the beginning of monitoring in 1986 to 2013 (more than 470 citizens of Slovak republic in 2013) although in the last years new infections increased (WHO 2013; Stanekova 2015). Several the HIV studies have been conducted (Hainova *et al.* 2014; Namulanda 2013;

Krcmery *et al.* 2013; Augustin *et al.* 2008). The trend of HIV/AIDS incidence in the population is rising also in the Slovak Republic and in the Czech Republic (Stanekova 2014; Hulinsky & Hamplova 2013). In 2013 there were 82 newly diagnosed patients in Slovakia, the majority of patients was infected HIV-1B subtype (Stanekova 2014).

One of the health problems among HIV-infected patients are the opportunistic neuroinfections. Cerebral toxoplasmosis is the most common neurological opportunistic disease manifested in HIV infected patients (Meira *et al.* 2013). Cryptococcal meningitis is the second most common cause of the opportunistic neuroinfections (Munivenkataswamy *et al.* 2013). In the pediatric population, these complications frequently have their

own unique disease identity, which may be related to maturational patterns evident in the developing brain (Wilmshurst *et al.* 2014).

The diagnostic possibilities of the Human Immunodeficiency Virus – HIV began to develop at its first successful isolation which led to a small step to the first commercial tests available in 1985. In diagnosing the presence of HIV infection, we focus on proof, or direct evidence or circumstantial proof of infectious agent or its parts. The most common method routinely used is ELISA, aimed at the detection of antibodies against HIV. In most cases, both types of antibodies are examined, namely HIV-1 and HIV-2 (Shors 2009; Zima 2013). Besides the antibody anti-HIV, the p24 viral antigen is detected in the blood of the patient, which is found as a soluble protein in the serum, detectable in the blood three weeks after infection. The detection of the p24 antigen was a shift in the early diagnosis of HIV, its designation contributing to a shortening in the window period of diagnosis (Zima 2013; Vetter *et al.* 2014).

Whenever there is a positive or uncertain result, it is necessary to continue further in the diagnosis with a confirmatory method called the Western blot, otherwise known as Immunoblotting. This step is a must because a positive ELISA does not necessarily mean the actual presence of HIV infection in a subject. The Immunoblot method indicates the presence of specific antibodies against various HIV proteins and glycoproteins (Kralova & Svecova 2012).

Besides the above mentioned indirect methods, the direct method of demonstrating the presence of viral nucleic acid RNA or the provirus DNA HIV are often less used. Such methods include the most common Polymerase chain reaction PCR, or a modification of the reverse-transcription Real Time PCR – PCR, which uses reverse transcription and the product is monitored in real time (Chun *et al.* 2014; Sanders *et al.* 2014).

For proper indication of treatment and the condition of the patient, a designated sub-population of T-lymphocytes, CD4 cells is added to the blood in the rest of the tests, and its ratio added to the CD8 lymphocytes.

The method which proves most reliable in detecting the presence of the HIV virus must be highly sensitive and specific. That is the reason why diagnosis uses a combination of several methods. Thus, we can summarise that the diagnosis of the immunodeficiency virus is divided into several categories. They are:

- Detection of antibodies;
- Identification of antigen;
- Detection of viral nucleic acid;
- Monitoring T-cell levels.

ANTIBODY DETECTION

Tests that detect the presence of antibodies with specific orientation of the HIV antigen are based on antigen-antibody reactions and thus the creation of immune complexes. The antibodies anti-HIV begin to develop

in the patient within three weeks, or up to three months after infection. It may also be the reason for misleading negative results at a time when the patient has not yet begun to produce antibodies. For verification of proper testing evaluation, it is necessary to repeat the test again after 6 months.

In the majority of people (90%), it is possible to detect the presence of antibodies three months upon being infected by the HIV virus at the latest. Today's tests can detect them earlier. The period during which the patient is newly infected, however, a seroconversion has not yet occurred, that is, the development of corresponding antibodies which are referred to as a so-called diagnostic window. During this period, it is not possible to detect infection in a subject by any kind of test for detecting antibodies (Kucirka *et al.* 2011; Menon & Kamarulzaman 2009).

THE ELISA METHOD

A highly sensitive, inexpensive and simple method is the ELISA (Enzyme-Linked Immunosorbent Assay). It is used as a screening method particularly for its high sensitivity and simplicity. However, if the result is not clearly negative or positive, it is necessary to continue in the diagnosis with greater specificity. For diagnosis of HIV infection using ELISA, we refer to the use of indirect diagnostics. We search for antibodies against specific antigens. This concerns a group of antibodies, IgG and IgM, which are directed against HIV specific antigens, or viral proteins found on the cover of the particles of the virus (Hakre *et al.* 2012). Isolated antigens used in the ELISA assays are: gp120, gp41, gp36 and rp24 which belong to the HIV 1/2 type, but several variations and other antigens are also available: p17, p24, p31, p51, p55, gp160 and gp26 (Tiwari *et al.* 2013).

The principle of this method is to connect the antigen to the wall from the plastic material indenture of the microtiter plate, the antibody present in the sample is left to react in the cultivation process with the antigen bound to the wall of the indenture for a certain amount of time (approximately 30 minutes at 37–40 °C) and then the indenture is rinsed-out in order to remove any unattached components (Shors 2009).

After rinsing, we add an enzyme conjugate which binds to a specific antibody attached to a solid phase, e.g. to the antigen bound to the wall of the microtiter plate. After adding the enzyme conjugate, the mixture is rinsed again. The enzyme component causes a change in the whole complex to the coloured product of whose colour we are able to measure. The conclusion of the entire process is an evaluation of the results, thus the presence of specific antibodies by spectrophotometry using a spectrophotometer. A spectrophotometer specifically determined for the ELISA method is called an ELISA-reader. It enables measurement of colour reaction at different wavelengths and is adjusted for the possibility of deduction directly from microtiter

plates. Different colour changes, which are recorded by a spectrophotometer, are directly correlated with the concentration of HIV-specific antibodies in the sample. To determine the test results for a spectrophotometric analysis, a dimensionless, physical module of absorbance is used. This can be an amount of visible radiation absorbed by a substance, or by a sample (Bartunkova *et al.* 2011).

Nowadays, automatic devices such as ELISA processors are being used in laboratories, which contain a pipette block for the dilution of samples and their application on microtiter plates with the addition of their own reagents, a block for rinsing, incubation and deduction or for the evaluation of results (Bartunkova *et al.* 2011). Each supplementary commercial test has exact specified resulting reference values, which are compared with the control values.

The test can be performed on patients with positive infection 3–6 weeks after infection. In rare cases, antibodies can form after 12 weeks. During the diagnostic window, a negative false ELISA assay can result, but false negativity after this period is very rare. In addition to false negativity, we can encounter cases of false positive results with ELISA assays. Such a phenomenon may occur in a situation where different autoantibodies are present, the patient has recently received an influenza vaccine, or an illness is present in the liver. For the possibility of failure of this test, although it is unlikely, a confirmatory test should be performed. HIV diagnosis should never be based on the carrying out of one set of tests (Menon & Kamarulzaman 2009; Rifkin *et al.* 2012).

QUICK TESTS

Some of these commercially produced tests are available in pharmacies and are intended for home use. One advantage is the speed in obtaining results, as well as confidentiality in testing. Experts are often sceptical of “self-testing” at home because it can lead to improper performance of the test. Among professionals, the view most often encountered is that these types of tests for the diagnosis of HIV infection should be in the hands of an expert (Pant Pai *et al.* 2013). This can lead to improper handling of the pipette which is a part of the test. The person performing the test at home may not hold the pipette in the correct position or may not gather sufficient samples. In such a test, there is a greater risk of false negativity for untrained people (Ng *et al.* 2012). Manufacturers themselves state that they cannot rely on the accuracy or truthfulness of the result if this is not carried out by trained staff.

Concerning tests which detect the presence of antibodies anti-HIV in the blood and saliva of the patient, and we can provide the results within 30 minutes. The INSTI test, which is one of the most rapid, delivers results within 60 seconds (Slev 2012). A false result can also be recorded in the case that the test subject was

at a very early stage (the so-called “window period”) of infection when the antibody cannot yet be detected. Normally, this condition occurs approximately 3 weeks upon infection.

We can say that non-professional diagnostics in the home is not the place if we want to be sure that the testing was done correctly. On the other hand, this type of test is beneficial for the better screening of patients who suffer from infection because, on the basis of these tests, an individual may visit a specialised facility where an expert can make a diagnosis in time. In health care facilities, where these tests are carried out by professionals and well-trained staff, these tests are a great asset especially in emergency situations, for example, if there is an urgent need for blood transfusions, the need to admit patients into medical facilities, transplants or work accidents by nursing staff. In the case that the result appears positive, it is referred to as “pre-positive” and needs to be confirmed in a diagnostic laboratory (Mayer 2006).

It is important that the patient has been informed of the need to undergo examination at least three months afterwards. The provision of information is part of the pre-test counseling, which is absent in cases of domestic testing (Stanekova 2013). All performed rapid tests should be confirmed by additional testing. They can be used in most cases only as a primary or screening method.

CONFIRMATION TEST

A confirmatory test called the “Western Blot”, or Immunopijak reaction, is a test that is performed if the ELISA test appears to be vague or has confirmed the discovery of specific antibodies against HIV. The Western blot confirmatory test is the most widely used for the elimination of false positive results. It is generally considered to be the “golden standard” for confirming the diagnosis of HIV (Delaney *et al.* 2011; Shors 2009). In today’s modern laboratory, such a test should not be absent and it should also be a must that laboratory staff are properly trained and prepared to implement such a test when necessary.

The origins of these methods date back to 1975 when E. M. Southern described the transfer of DNA fragments from agarose gel to nitratecellulose membrane. The method was named after the author the “Southern blot”. In 1977, the method was improved by A. J. Alvin and he called the method the “Northern Blot”. In 1979, J. Renart and H. Towbin adapted the method and used it to analyse proteins which is known as Immunoblotting. W. to N. Burnett came up with a new idea and used it to detect protein-specific antibodies marked by radioactive “protein A”. The method received its new name, “Western Blot” (Bartunkova *et al.* 2011). With the aid of this method, we are able to demonstrate the presence and identify specific proteins from a full range of various others where there are extracted cells.

Put simply, this method can be divided into a group of three basic steps. The first step is the separation of proteins by size, followed by their transfer to a solid support, and finally the designation of the target protein by reaction with a specific antibody. The result is a visualisation of the interaction between a protein and antibodies (Mahmood & Yang 2012).

Proteins, whose presence and interaction with specific antibodies we wish to prove, are from a group of nuclear, or “gag” (p13, p18, p24, p55) as well as the covers of proteins, or “env” (gp160, gp120, gp41). The antibodies present against the Gag group indicate the presence of acute infection and their levels of gradual transition of infection to the latent stage decrease, the antibodies against the Env group are present on a longterm basis, even during a silent period (Rajčáni & Čiampor 2006; Tiwari *et al.* 2013).

The principle of the Western Blot is the separation of viral proteins (commercially available) gel electrophoresis processes, the transport of the nitratecellulose membrane where reaction occurs with the test serum of the patient. The aim is to find out whether or not the proteins react at all or whether they are recognized by the patient’s antibodies (Mahmood & Yang 2012; Shors 2009).

The antigens which were classified in the electrophoresis process are divided into individual nitratecellulose membrane fractions. The advantage is that it is possible to detect a number of antibodies against specific antigens simultaneously in one sample (Bartůňková *et al.* 2011).

The nitratecellulose membrane, following the binding of proteins, is shredded into strips and left to react with the patient’s serum, with positive serum containing antibodies and also with negative serum which does not contain any antibodies. Both positive and negative sera are used as controls. If there are antibodies present in the patient’s serum, they will react with antigens or viral proteins.

The entire reaction process is then rinsed in order to remove any antibodies which did not react. In the next step, we will add specific secondary antibodies called anti-human IgG with conjugated enzymes that produce a colour reaction when recognised by the antibody (anti-HIV) and achieve their visualisation (Gallagher *et al.* 2008; Mahmood & Yang 2012). For unclear results of the test, it is recommended to repeat the test after 3–4 weeks or use PCR.

IDENTIFICATION OF P24 ANTIGEN

An important method in diagnostics is the identification and detection of viral capsid p24 antigen levels. Method for the detection of p24 antigen is progress in early diagnostics of HIV infection, particularly acute infection and also could be the commonly used method in future in the diagnostics of children, who are born to HIV positive mothers (Wessman *et al.* 2012). P24 antigen is detectably present in the serum of the patient

between the first and third week of the infection. Therefore it can be detected even during the “window period” during which there will be no more seroconversion antibodies and can not be captured (Zima 2013; Menon & Kamarulzaman 2009; Wetter *et al.* 2014).

In addition to early diagnostics of p24 antigen we can find it also because the positive ELISA test and negative Immunoblot test and also in patients in which the likelihood of infection is high, have symptoms, but the ELISA test was negative. The earlier versions of the test for p24 detection had low rates of specificity, but these limitations were largely eliminated and on the present the sensitivity and specificity correlate with the analysis on the principle of the PCR (Wessman *et al.* 2012).

THE FOURTH GENERATION OF SCREENING TESTS

Fourth generation tests for the diagnostics of HIV infection simultaneously detect the presence of HIV antibodies and p24 antigen. They are recommended for excellent values of sensitivity and specificity (Gökengin *et al.* 2014). This generation of tests is used in practice only the last decade, in comparison with older generations provide higher sensitivity. The test is very reasonable not only from the view of diagnostics but also from the view of epidemiology. Thanks to the simultaneous evaluation of p24 antigen it is possible to capture the early stages of infection (Slev 2012). The Individuals who are in this stage of the disease, often do not know about your diagnosis and significantly contribute to the spread of the disease.

The use of fourth generation tests reduces the duration of the diagnostic window for 5 days and provides the possibility of early diagnostics of acute HIV infection, however, the detection of nucleic acids remains method with the highest sensitivity (Slev 2012). In addition, the advantage is lower costs, less the number of trained laboratory workers and less time in comparison with the application of such tests individually. It is very likely that these tests will be, thanks to benefits which provils in the diagnosis introduced and used as standard or routine part of the diagnostics of HIV infection (Nasrullah *et al.* 2013).

DETECTION OF VIRUS NUCLEIC ACID

In the event when it is not possible to capture the presence of specific antibodies against the virus, it is necessary to apply the tests, which are not based on the formation of immune complexes. Therefore we perform DNA PCR method for identifying a viral DNA integrated into the host cell, or PCR designed for quantification of RNA respectively viral load, which is an important information for antiretroviral therapy indication.

PCR method is also suitable in cases of testing children of HIV-positive mothers. It is necessary to

determine the presence of infection, as soon as possible, in particular due to the initiation of antiretroviral therapy, which could prevent the development of a long-term infection (Mitchell *et al.* 2014).

POLYMERASE CHAIN REACTION AND ITS PRINCIPLE

The principle of PCR method is amplification of a specific segment of the polynucleotide chain *in vitro*. Amplification is carried out thanks to the enzymatic activity of the DNA-polymerase, which extend the chain. Increasing number of copies is caused by a continuous repetition of whole process. By this mechanism can be within a few hours to get millions of copies of a specific fragment. Each reaction mix for PCR contains:

- Deionized and sterile water
- PCR buffer, which is needed for the stability and activity of DNA-polymerase
- Magnesian cations, which are cofactor of DNA polymerase
- Deoxyribonucleosidtriphosphates serving as a building material for copying of resulting chain.
- Primers
- DNA polymerase
- A template, that is to say a sequence with genetic information.

The reaction is controlled by cyclic varying (the 20–40 cycles) of different temperatures at PCR cyclus (Mahato 2011; Votava 2010). At every temperature the other phase of the reaction takes place and so reaction shall be divided into the following basic steps:

- The temperature above 90 °C – thermal process of DNA threads denaturation
- The temperature of from 45 °C to 65 °C – phase annealing, respectively annealing at “his place” in the template DNA. The temperature at which this stage of the reaction takes place depends on the length of the primers.
- Temperature 72 °C – polymerization occurs. DNA polymerase starts working and by building particles dNTPs to synthesize new complementary strand of DNA.
- At the end of this phase from all PCR runs longer than the other cycles, because of the final completion of each polymerization, which is ongoing (Mahato 2011).

Various PCR methods have been proposed to help detect the presence of a specific segment of the HIV genome, HIV GAG gene (Menon & Kamarulzaman 2009; Jilich *et al.* 2014). In clinical practice, most commonly used method is Real-Time Reverse Transcriptase PCR, which evolved from the classical PCR method, or its modification. It is widely used for prediction and prospective diagnostics, in particular in relation to the quantification of the viral RNA (Kiselinova *et al.* 2014).

The method uses an enzyme reverse transcriptase for the transcript to a working copy of the cDNA, which is consequently amplified. To view of product and its quantification are using fluorescent dyes binding to the nucleic acid and fluorescence enhancing is correlated with increasing of the product, which quantum is determined on the basis of the calibration curve. Another option is use of the spectrophotometric sample analysis, or measuring of its absorbance (Sanders *et al.* 2014).

Quantification of nucleic acids is used both for early diagnostics of infection, because it decreases the period of diagnostic “window”, but it also serves for confirmation of uncertain results obtained from the serological methods. Determination of HIV RNA has a justification in newborns, who were exposed during prenatal development, due to an early diagnostics of infection (Wessman *et al.* 2012).

In children who are born HIV-positive mothers, the number of presented HIV RNA copies is detected directly in umbilical blood as well as in samples that are collected during 1, 3, and 6. month after birth (Zima 2013). Quantitative tests demonstrate the level of circulating vRNA copies in 1 ml of blood, called as the vRNA. viral load (Jilich *et al.* 2014).

Using the value of viral load we can evaluate the status of infection in the patient. The value of viral load is determined from the sample of blood plasma from a patient, where we search HIV RNA (Gökengin *et al.* 2014). The determination of viral load value is essential for the correct indication of antiretroviral therapy, and also for obtaining of information about its effectiveness (Zima 2013). It is determined at the same time with determining of CD4 cells counts (levels). Antiretroviral therapy can significantly reduce the number of circulating copies (Menon & Kamarulzaman 2009).

Quantification of HIV DNA refers to the number or representation of cells that are latently infected (Chun *et al.* 2011). HIV is stored in CD4 T-lymphocyte cells in the form of proviral DNA which occur in peripheral blood as well as in cells associated with the lymphatic tissue (Chun *et al.* 2011). Proviral DNA may be present in cytoplasm of host cells or may be a part of her chromosomal DNA, in which have already left incorporate.

Negative DNA PCR test after the outcome of the confirmation test may indicate that the initial ELISA test has failed and it is about his false positivity. The reason may be related with the structure of recombinant antigens or other components of the test and with the unique properties of the patient serum (Jilich *et al.* 2014).

VIRUS ISOLATION

Isolation method is do not used in clinical practice because it is labour intensive and protracted. We meet her in the specialized laboratories and carries out mainly for scientific purposes. It is used mixed culture of blood mononuclear cells of the patient and lympho-

cytes of healthy donor that is stimulated by phytohemagglutinine and interleukin-2 (Votava *et al.* 2010). However, most often is it circumvented by PCR methods (Wessman *et al.* 2012). Cultivation of a virus is a costly diagnostic method so its exertion cannot be used. Electron microscopy is also very expensive (Kralova & Svecova 2012).

MONITORING OF T-LYMPHOCYTES LEVELS

HIV infection directly affects the patient's immune system so it is necessary to evaluate his immune system periodically or monitor, respectively. In order to evaluate the state of patient, it is necessary to investigate the level of CD4 T-lymphocytes (Mbopi-Kéou *et al.* 2012). During advancing progress of HIV infection, CD4 cell count changes. Based on the monitoring of CD4 count, we can estimate the stage in which the infection is, and we can also suggest a therapeutic approach (Koyalta *et al.* 2013).

Evaluating of the immune status of HIV positive patient each rate the number of specific T cells, which bear the CD4 mark. CD4 lymphocytes are white blood cells and with regard in their function in immune system are also called auxiliary or helper lymphocytes (Hořejší *et al.* 2013). They recognize disturbers in the body, such as the HIV viruses, and give signal to the immune system to defend against infection. They play an important role in HIV infection as the host cells for the virus. HIV is incorporated into the CD4 cells genome and by the proliferation is created a number of subsequent populations of infected cells. The infected CD4 cells synthesize many HIV virions. CD4 lymphocytes multiply in order to prevent the infection, but since they are infected, only allow the production of additional copies of the virus.

The number of CD4 cells is not constant and depends on a number of factors, such as gender, smoking, immune status, and genetic factors also environmental, demographic factors (Malaza 2013) and even variations during the day occur. For these reasons, to obtain relevant data, sometimes it is recommended to repeat determination of CD4 cells count (Hansen *et al.* 2013).

For different values we can predict various stages and adjust drug therapy, not only antiretroviral therapy (WHO 2013). The World Health Organization created a classification system revised in 2007 according to which the clinical picture of the patient is divided into four stages:

- first stage (acute phase of infection);
- second stage: it begins to manifest signs of deterioration of the immune system;
- third stage: the state, when it is appropriate to consider an indication of antiretroviral treatment (in particular, when the CD4 cells count in blood dropped below 500 per cubic milliliter);

- fourth stage (a synonym is an AIDS, syndrome which leads to the exit of the organism): the number of CD4 cells (cells count) gradually decreases to the level approaching/nearing zero.

After HIV-infecting the CD4 cell count usually rapidly decreases and then stabilizes at a level of around 500. Later, the rapid decrease occurs about a year before the outbreak of AIDS patient. Then the level is monitored every 3 months. Below the limit 350 cells per cubic millimeter dramatically increases the risk of serious illnesses. Antiretroviral therapy will allow the return of the original value or at least improving of the levels of CD4 lymphocytes and so protects the patient's immune system (Yeh & Coen 2012).

Many HIV infected patients are at risk for HTLV-I co-infection worldwide. These patients exhibit abnormally high CD4+ T lymphocyte counts that are not a reliable parameter of the immune status. Santos *et al.* published results from the study in HIV/HTLV co-infected patient who developed progressive multifocal leukoencephalopathy despite of a high CD4+ T lymphocyte count emphasizing that this situation can be observed in regions around the world where HTLV-I infection is prevalent (Santos *et al.* 2013).

DETERMINATION OF THE CD4 T-LYMPHOCYTES LEVEL

For determination of CD4 T-lymphocytes number we use mainly the method of flow cytometry. We assess not only the number, but also their proportional (percentage) representation. In the flow cytometry is applied the principle of light scattering on the basis of particle size distribution or granulation of the cells passing through the laser beam and also the cell fluorescence after labeling with specific cells labeled with specific monoclonal antibodies, that are binding to specific cell surfaces (Pattanapanyasat *et al.* 2007). We are using so-called the discriminatory power of antibodies which are directed oriented against specific cell receptors, such as especially CD4 cells (Malaza *et al.* 2013). Routine determination of CD4 T-cells requires significant financial expenses, mainly due to the high price of the device. High financial cost are making the flow cytometer for countries with low levels of the health-care system almost inaccessible.

An alternative solution comes in the form of equipment, the so-called. Pima CD4 Analyzer. Pima CD4 is cheaper, easily portable and easily handling device that allows the determination of the absolute number of CD4 T-lymphocytes from the capillary or venous blood (Manabe *et al.* 2012; Wade *et al.* 2014). It is based on the reaction fluorescent labelled antibodies with surface antigens CD3 and CD4. The result is evaluated by using an integrated camera and evaluation device and is displayed directly on the screen (Manabe *et al.* 2012).

Although less accurate, but relative good results, PIMA CD4 demonstrate results which adequately correlate with the results provided by the flow cytometry. The PIMA CD4 device provils an attractive possibility how to reliably determine, whether it is appropriate for the patient to begin with antiretroviral therapy and prevent delays in treatment. Especially in areas where the possibilities of diagnostics linked with determination of CD4 T-lymphocyte limited (Manabe *et al.* 2012; Wade *et al.* 2014).

CEREBRAL DISEASES

Only few articles related to opportunistic neuroinfections can search out in Medline in the last decade (2005-2015). Cerebral toxoplasmosis is the most common neurological opportunistic disease manifested in HIV infected patients (Meira *et al.* 2013). Toxoplasma encephalitis is caused by the opportunistic protozoan parasite Toxoplasma gondii. Primary infection with T. gondii in immunocompetent individuals remains largely asymptomatic. In contrast, in immunocompromised individuals, reactivation of the parasite results in severe complications and mortality. Molecular changes at the protein level in the host central nervous system and proteins associated with pathogenesis of toxoplasma encephalitis are largely unexplored (Sahu *et al.* 2014). Excretory/secretory antigens (ESA) are serological markers for the diagnosis of reactivation of the infection in HIV-infected patients with cerebral toxoplasmosis. Immunosuppressed patients develop high antibody titers for ESA. However, little is known about the humoral response for these antigens (Meira *et al.* 2013). Data from those authors suggest that IgG4 can be valuable for supporting the diagnosis of focal brain lesions, caused by T. gondii infection, in HIV-infected patients. This approach might be useful, mainly when molecular investigation to detect parasites is not available.

Cryptococcal meningitis is mainly caused by members of the *C. neoformans/C. gattii* species complexes. The growing place of the neuromeningeal cryptococcosis in the neuromeningeal opportunist pathology of HIV positive patients was suggested by authors from teaching hospital in Dakar (Soumaré *et al.* 2009). Cryptococcal meningitis has emerged as a leading cause of the infectious morbidity and mortality in HIV seroreactive subjects and it is the second most common cause of the opportunistic neuroinfections in it. As this is a indistinguishable from other causes of meningitis, its early diagnosis is the key to the therapeutic success (Munivenkataswamy *et al.* 2013).

Shankar *et al.* in their overview (2005) from Indian scenes concluded that neurological manifestations of HIV infection and AIDS are being recognized with a frequency that parallels the increasing number of AIDS cases. Next to sub-Saharan Africa, India has the second largest burden of HIV related pathology, essen-

tially caused by HIV-1 clade C in both the geographic locales, in contrast to USA and Europe. But the true prevalence of HIV related neuroinfections and pathology is not available due to inadequate medical facilities, social stigma and ignorance that lead to underdiagnosis. Neurotuberculosis, followed by cryptococcosis and toxoplasmosis in various combinations are the major neuropathologies reflecting the endemicity and manifesting clinically by reactivation of latent infection.

Kiwou *et al.* published descriptive study in which analysed 8,709 patients with tropical neuroinfections in south sudanese rural hospitals (Kiwou *et al.* 2013). The most frequent parasitic neuroinfections were cerebral malaria, sleeping sickness, trypanosomiasis, schistosomiasis, toxoplasmosis. Authors concluded that decreased incidence of serious neuroinfections in Sudan may be related to improvement of effective therapeutic options, represented by (i) intermittent preventive therapy (IPT) for malaria, (ii) by suppression of sleeping sickness vectors and (iii) by better accessibility of antibiotics.

FUTURE IN HIV DIAGNOSES

Guralla *et al.* published study in which was developed a novel complementary metal-oxide semiconductor (CMOS) chip based, pH-mediated, point-of-care HIV-1 viral load monitoring assay that simultaneously amplifies and detects HIV-1 RNA (Guralla *et al.* 2016). A novel low-buffer HIV-1 pH-LAMP (loop-mediated isothermal amplification) assay was optimised and incorporated into a pH sensitive CMOS chip. The produce a point-of-care device which would be of benefit in resource poor regions, and could be performed on an USB stick or similar low power device).

The future perspective in HIV diagnostic are biosensors. The principle of diagnosis is based on molecular interaction between the surface linked oligonucleotides and hybridization with complementary target nucleotides present in the sample. Biosensor is an upcoming technology and can be widely used for the molecular diagnosis of HIV. The present method of HIV diagnosis is under numerous negative factors. They are non-confirmatory, which requires further other confirmatory tests to stamp the presence of infection. Biosensor technology offers several benefits over present diagnostic tools, such as, specificity, simplicity, rapidness, continuous monitoring, less expensive and easy in portability of the instrument (Arya *et al.* 2015).

Portable tests for HIV monitoring comprise CD4+ T-cell count assays and HIV quantification assays. Currently, only a handful of truly portable CD4+ T-cell counters are available commercially: PointCare NOW, the CyFlow miniPOC (Partec), Pima Test (Alere) and Daktari CD4+ (Daktari Diagnostics). All are fully automated, can be powered by batteries or electricity, use ≤ 25 μ L of blood and provide same-day results. Additional POC CD4+ T-cell tests in development include

a multiplex infectious disease test (MBio Diagnostics) and a semiquantitative, electricity-free CD4+ T-cell blood test (Zyomyx) (WHO 2012).

CONCLUSION

In the fight with the global health problem such the epidemic HIV infection is no doubt, plays a key role a simple, reliable and fast diagnostics. This has its own specifics, laboratory, psychological and social. At the present time, HIV infection is treatable, but the disease remains incurable. Modern diagnostic methods, which make the infection detectable already at an early stage, directly correlate with successful treatment. Despite of significant advances in the diagnostics and treatment of HIV infection, this disease is still unable to get completely under control.

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