



AmpliSens
biotechnologies

HIV Infection



Reagent Kits Format and Composition

By detection type:

FRT format – real-time fluorescence detection

The format is intended for use of specialized equipment for real-time PCR. Labeling of reagent kits reflects the adapted equipment:

RG — Rotor-Gene 3000/6000 (Corbett Research)

iQ — iCycler/iQ5 (BioRad)

Mx — Mx3000P/Mx3005P (Stratagene)

HELISA format – hybridization enzyme linked immunosorbent assay

The format is intended for amplification in a standard thermal cycler with subsequent detection through hybridization accumulated in the course of labeled DNA PCR with a specific probe sewn to the bottom of the tray cavity. Detection of the signal is achieved by a method similar to the one used for ELISA assay: sample solutions containing DNA fragments to be determined change their color as a consequence of the substrate destruction by alkaline phosphatase.

SEQ format - detection by sequence analysis

The format is intended for detection with use of Beckman Coulter and Applied Biosystems sequencers

By configuration:

Complete Set Reagent Kit format

The kit includes reagents for extraction, amplification and detection.

Amplification Reagent Kit (PCR Kit) format

The kit includes only amplification reagents.


Reverse Transcription and Amplification Reagent Kit format


The kit includes reagents for reverse transcription and amplification.

By hot start type and filling:

«Wax» format


Is provided by a wax layer:

 The kit includes PCR test tubes ready for use with a lower mixture applied under wax;

 The kit includes vials with reagents not dispensed into PCR test tubes.

“Hot-Start” format

“Hot Start” is ensured by modified polymerase activated at heating (TaqF):

 The kit includes vials with reagents not dispensed into PCR test tubes, modified TaqF polymerase is used

As compared to PCR test tubes ready for use with a lower mixture applied under wax this format improves the quality of the «hot start» and quality of results without increasing the associated labour intensity. On preparation to PCR test all components are pre-mixed and then the reaction mixture is dispensed into PCR test tubes once.



FRT Format Complete Set Reagent Kits



«RIBO-sorb» and «HEM-sorb» Reagent Kits for RNA/DNA isolation

- Lysing solution
- Sorbent (silica)
- Washing solutions
- Eluting solution

Reagent Kits for Reverse Transcription and Amplification

- PCR-mixture-1 (primers and probes)
- PCR-buffer solution
- Modified polymerase TaqF
- M-MLV revertase (only for RNA detection)
- Positive control samples (PCS) of the isolation stage and PCR, calibrators (standards)
- Negative control samples of the isolation stage and PCR (NCS)
- Internal CS (control sample) of the isolation stage and PCR (ICS)



HELISA Complete Set Reagent Kits



Reagent Kits for Nucleic Acids isolation:

RNA-“RIBO-sorb”

- Lysing solution
- Sorbent (silica)
- Washing solutions
- Eluting solution (to be stored at -20°C)

DNA-“Cytolysin”

- Cytolysin
- Hemolytic
- Proteinase K solution

Reagent Kits for Reverse Transcription and Amplification

- PCR-mixture-1 (primers and probes)
- RT-PCR HIV buffer solution
- Enzymes for reverse transcription and amplification (AMV, Taq, to be stored at -20°C)
- Positive control samples (PCS) of the isolation stage and PCR
- Negative control samples (NCS) of the isolation stage and PCR
- Internal control sample (ICS) of the isolation stage and PCR
- Mineral oil

Reagent Kit for HELISA analysis

- A tray sensitized by DNA-probes (to be stored at -20°C)
- Denaturing agent
- Hybridizing buffer solution
- Conjugate buffer solution
- Conjugate
- Washing buffer solution
- Stopping solution
- TMB substrate

HIV Infection

At the present time the epidemiological situation with regard to HIV infection in Russia is extremely unfavourable. As of the end of 2007, more than 430 thousand people were affected by HIV infection.

Depending on structural and antigenic properties two virus types are differentiated: HIV-1 and HIV-2. HIV-2 occurs considerably less often than HIV-1. In accordance with 1991 Nomenclature, there are three independent HIV-1 groups: «M» (main); «O» (outlier); «N» (non-V/non-O). In addition to this, there are the so-called "circulating recombinant forms (CRF)" viruses with a mosaic structure of the genome, elements of which are typical for representatives of various subtypes. Groups O and N are less widely spread and occur in African countries population. Group M includes 11 subtypes: A1, A2, B, C, D, F1, F2, G, H, J, K. Transmission ways of the virus are very important for the virus spread. HIV is transmitted by three ways: at heterosexual and homosexual sexual intercourse, parenterally with blood and blood products and vertically: from the infected mother to the child by an intrauterine way, during the child delivery or soon after the childbirth at breast feeding.

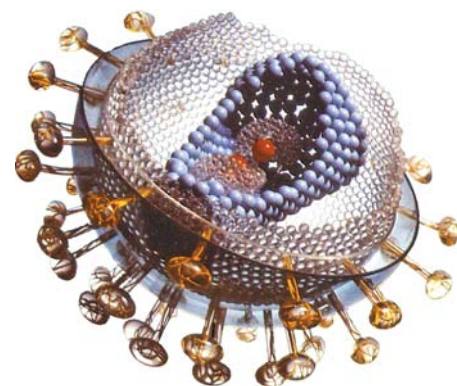
In the middle 1990-s variants of 8 HIV-1 subtypes were circulating in the USSR (from A to H), subtype B being the predominant. In the Russian Federation quick spread of the infection started in the second half of 1996 when an outburst of the HIV-infection occurred in intravenous drugs consumers in Kaliningrad, Tver, Novorossiysk, Saratov and Nizhniy Novgorod. The outburst was caused by HIV subtype A that was quickly spread among narcotic drug dependent persons and then among their sexual

partners. Today the prevailing majority of HIV infection cases in Russia are connected with subtype A of the virus.

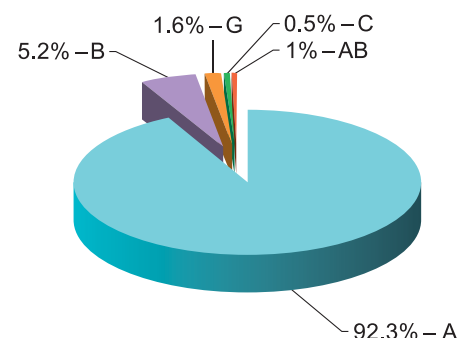
HIV-1 Vital Cycle

A particle of the virus entering the blood circulation attaches itself by a superficial gp120 glycoprotein to CD4 cell receptors. This results in gp120 conformation, at the same time the second part of gp120 intended for binding to co-receptor (CCR5) becomes available for interaction. Interaction of CCR5 with the CCR5-binding part of gp120 promotes change in the gp41 protein conformation, resulting in conjugating of surfaces of the cell and the virus particle. Following the conjugation RNA encircled by nucleocapsid and capsid proteins enters the cell. Then RNA enters the cell cytoplasm as a component of the reverse-transcriptase complex. The double stranded DNA - a product of the reverse transcription reaction - is transported in the cell nucleus and then is integrated in the DNA of the host cell. The DNA of the pro-virus becomes the matrix for transcription resulting in RNA formation that gives rise to a new virus particle.

HIV-infection is characterized by a continued course, clinically associated with progressive immunity deterioration leading to development of grave forms of opportunistic diseases. Many investigators sought to reflect the course of HIV infection in the form of clinical classifications.



Distribution of HIV subtypes in Russia



Central Scientific and Research Institute of Epidemiology of the Ministry of RF, 2003

Russian HIV-infection Classification (version 2002)

1. Incubation stage

2. Primary manifestations stage

- 2A Asymptomatic
- 2B Acute HIV-infection without secondary diseases
- 2C Acute HIV-infections with secondary diseases

3. Subclinical stage

4. Secondary diseases stage

4A Weight loss by less than 10 percent; fungous, viral, bacterial affection of skin and mucous tunics; herpes zoster; repeated pharyngitis, sinusitis.

Phases: advance (on the background of absence of antiretroviral therapy and on the background of the antiretroviral therapy); remission (spontaneous, after the earlier conducted antiretroviral therapy; on the background of the antiretroviral therapy).

4B Body weight loss by more than 10 percent; unexplainable diarrhea for more than one month; villous leukoplakia; tuberculosis, repeated or resistant viral, bacterial, fungous, protozoan affections of internal organs; repeated or disseminated herpes zoster; localized Kaposi's sarcoma.

Phases: advance (on the background of absence of antiretroviral therapy and on the background of the antiretroviral therapy); remission (spontaneous, after the earlier conducted antiretroviral therapy; on the background of the antiretroviral therapy).

4C Cachexia; generalized and viral, bacterial, fungous, protozoan and parasitic diseases: pneumocystic pneumonia; candidosis of oesophagus, bronchi, lungs; atypical micobacterial diseases; disseminated Kaposi's sarcoma; affections of the central nervous system of various etiology.

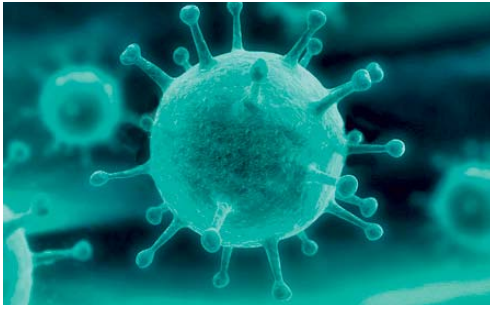
Phases: advance (on the background of absence of antiretroviral therapy and on the background of the antiretroviral therapy); remission (spontaneous, after the earlier conducted antiretroviral therapy; on the background of the antiretroviral therapy).

5. Terminal stage

HIV-Infection Pathogenesis

The 4 most important pathogenic mechanisms of HIV-infections among the numerous others include:

1. absence of virus elimination from the organism after the primary infection;
2. persistent viral replication in lymphoid organs within the whole period of HIV-infection;
3. chronic stimulation of the immune system provoking inadequate immune activation and progressive deterioration of the immune response;
4. destruction of the lymphoid tissue leading to important impairment of the sustainable ability to maintain HIV-specific immune response and generation of immune response to other pathogens.



Diagnostics of HIV-infection is carried out by complex assessment of epidemiological data, results of clinical examination and laboratory findings. The decisive role in establishment of HIV-infection diagnosis belongs to laboratory diagnostics that consists in detection of antibodies to HIV in blood by ELISA analysis (enzyme-linked immunosorbent assay) with subsequent confirmation of positive results by immunoblotting (IB). The effectiveness of infected patient detection by such method of HIV-infection makes 99 percent. But serologic diagnostics is not effective in the period of the so-called «serologic window» when in the first days following the contamination antibodies to HIV can't be detected by ELISA method due to their absence or low concentration. In addition to this, the effectiveness of diagnostics by detection of antibodies to HIV is reduced to nil at examination of children born from HIV-infected mothers as non-infected children might have antibodies to HIV in blood within a long period of time after birth (for a year or more).

Another drawback of the serological diagnostics is falsely positive results, that's why a more specific test – IB – is required for confirmation of diagnosis that is rather expensive (the cost of one IB makes 20-40\$). In addition to this, the IB method might produce dubious results. In accordance with the evidence obtained by foreign investigators the percentage range of equivocal results makes from 4 to 20%. Equivocal results of IB leads to a high price of diagnostics and delay in diagnosing the case (up to 3 months and longer).

The above-listed disadvantages of ELISA diagnostics set one thinking about its supplementation and in some cases about its replacement by methods of direct virus detection.

One of the most effective current methods of direct HIV detection is specific amplification of nucleic acids in vitro, in particular, its most developed variant – a method of polymerase chain reaction (PCR). This method has a lot of advantages. First of all, detection of virus DNA/RNA allows reducing the length of the «serological window» by 11 days at the average. Secondly, the low cost of Russian PCR-tests (3-5\$) is an optimum solution of decoding of equivocal IB results, and subsequently – possibly a complete replacement of the expensive IB method. Thirdly, PCR method became an indispensable approach for HIV-diagnostics in children born from HIV-infected mothers

HIV-Infection Diagnostics

HIV-infection diagnostics in children

born from HIV-infected mothers is difficult due to the fact that mother's antibodies to HIV persist in such children's blood for a long time. But not every child born from the infected mother is infected with HIV, in spite of the fact that children are subjected to high risk of HIV infection in the intrauterine period, during the delivery and breast feeding. If no preventive measures are taken, the risk of mother-to-child HIV transmission in children on bottle feeding makes 15-30 percent; breast feeding increases the risk up to 20-45 percent. Today effective measures intended at prevention of vertical HIV transmission are developed: antiretroviral therapy that is administered to mother during the pregnancy and childbearing and to the child on the first weeks of life; obstetrical interventions, including Caesarian operation; refusal from breast feeding. If these methods are available and applied, the incidence of mother-to-child HIV transmission can be reduced to 1-2 percent.

Recommendations effective in Russia suggest that making or disposing of the HIV diagnosis to children born from

HIV-infected mothers should be carried out on the basis of serological analysis results not earlier than at the age of 18 months.

The problem of earlier HIV-infection diagnostics

in newborns was solved abroad several years ago with development of molecular-genetic methods that allow detection of HIV genome fragments in the peripheral blood at early contamination stages. The evidence was obtained that the HIV provirus DNA is determined by the age of one month in the majority of children and practically in all - by the age of 6 months. Based on these data it's recommended to conduct the polymerase chain reaction (PCR) for HIV provirus DNA or for HIV RNA for the first time within 48 hours after birth. The umbilical blood is not fit for examination due to possible content of mother's blood in it. The second examination for HIV provirus DNA or for HIV RNA is carried out on the 6-8th week of the child's life irrespective of the result of the first examination. The final decision about HIV-infection presence in child is made not later than the age of 6 months of the child.

Infectious safety of donor blood

Blood screening with serological methods in the majority of cases allows prevention of HIV-infection transfer at blood transfusion. But the problem of «serological window» remains when the presence of specific antibodies is not detected but the virus is actively proliferating in the infected body. Since 1999, in the US and countries of the Western Europe samples of donor blood are analyzed for RNA of HIV virus and Hepatitis C by molecular-genetic tests that are widely used. Currently FDA has

approved test-systems for donor blood screening for RNA of HIV and Hepatitis C. Since 2008, PCR method for testing of donor blood is brought under regulation in the RF. «AmpliSens®» reagent kits for detection of HIV and Hepatitis C RNA and Hepatitis B DNA passed testing in L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute and are licensed for production and application in the territory of the RF.

HIV-Infected Patients Monitoring

The most important area of HIV diagnostics molecular method application is determination of HIV RNA in the blood plasma. In accordance with up-to-date requirements determination of human immunodeficiency virus (HIV) RNA in the blood plasma («viral charge») is an obligatory procedure, starting from the moment of the HIV-infection diagnosis making, as well as on the stage of prescription of antiviral therapy and subsequent monitoring of the therapy effectiveness so far has been considered the major laboratory

indicator for evaluation of a possibility of HIV-infection progress. The huge accumulated experience shows clear dependence of the risk of the disease progress from the HIV RNA concentration in the blood plasma. That's why the first recommendations as related to start of the antiviral therapy were based on two laboratory findings: CD4-lymphocytes concentration and concentration of HIV RNA.

But with implementation of highly effective schemes of antiretroviral

therapy it turned out that the risk of AIDS development depends mainly on the level of CD4 leukocytes before the start of therapy. Thus, in accordance with current recommendations antiretroviral therapy is prescribed to HIV-infected patients on the basis of clinical manifestations and concentration of CD4 leukocytes.

Viral charge is the earliest indicator of success or failure of therapy that is nearly by a month in advance in detection of change in number of CD4 leukocytes. Statistically important change in viral charge is change in more than 3 times or by 0.5 lg. The first examination concerning viral charge after the initiation of the therapy should be carried out in 4-8 weeks. Virusological criteria of the therapy effectiveness are reduction of viral charge by more than 0.5 lg in 4 weeks and by more than 1 lg in 8 weeks of therapy. Failure of the viral charge to reduce to lower than 400 copies per ml by the 24th week of treatment or to the level lower than 50 copies per ml by the 48th week of treatment signifies an inadequate answer. If the viral charge once reduced to the undeterminable value and then the twice repeated measurement with an

interval 4-8 weeks showed that it again restored to determinable indicators, this may mean a risk of virusological ineffectiveness of therapy. In most cases this is the evidence of drug resistance of HIV and requires immediate change of the treatment regimen. Insignificant step-up of viral charge to the undeterminable level up to 50-200 copies per ml ("surges") might be registered without stable virus strains, but nevertheless this might become the reason to discuss issues of discipline with regard to therapeutic regimen with the patient.

Today there are several approaches to quantitative determination of HIV RNA: polymerase chain reaction (PCR) with reverse transcription; isothermal amplification (NASBA) and bDNA (branched DNA hybridization). These methods are widely used in the world and in Russia for determination of HIV RNA concentration in the blood plasma. "AmpliSens®" reagent kits are used for diagnostics of HIV-infection in the RF for more than 8 years.

HIV Drug Resistance to Antiretroviral Therapy

Antiretroviral therapy for HIV-infection has considerably increased quality and expectancy of life of HIV-infected patients. At present more than 20 drugs intended at inhibition of major virus enzymes are registered and licensed for use in the world. The majority of these drugs are divided in two classes: protease inhibitors and reverse transcriptase inhibitors. The latter are divided in nucleoside and non-nucleoside analogues. The most effective scheme of treatment is a combination of several drugs from various classes that affect the target simultaneously, promoting quick reduction of HIV RNA in blood plasma to the level undeterminable by modern tests. But in majority of patients this therapy becomes ineffective despite use of such highly effective treatment regimes as combined therapy by several drugs inhibiting various stages of virus replication. Though the reason of the failure of the drug to produce the desired effect might be multi-factorial, practically in all cases patients develop resistance to applied drugs.

Quick development of HIV resistance to antiviral drugs can be accounted for by the following reasons:

- high degree of HIV replication (up to 10¹⁰ new viral particles might appear in the body of the infected patient per day);
- high degree of HIV mutation (due to no revising activity of the reverse transcriptase of the virus, which provokes development of 1-2 mutations per 1 replication cycle);
- incomplete suppression of virus replication by antiviral drugs;
- therapy with drugs with a "low genetic barrier" with regard to development of resistance.

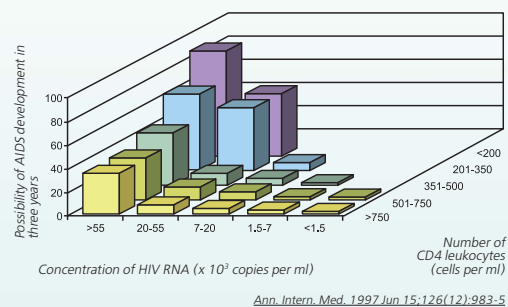
Determination of HIV drug resistance

There are two approaches to determination of HIV drug resistance: genotypic and phenotypic. Phenotypic tests evaluate the ability of the virus to grow in presence of various antiviral drugs in vitro.

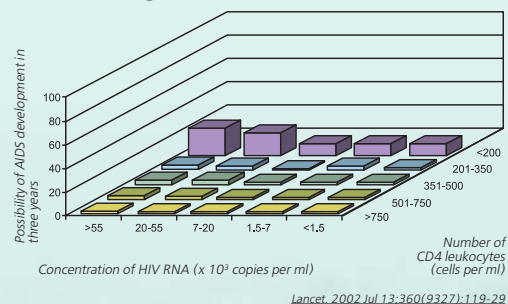
Genotypic tests determine specific mutations responsible for development of resistance.

Molecular-genetic methods of drug resistance determination based on DNA sequence analysis were widely spread due to a number of advantages: a relatively low cost, speed and simplicity of analysis. In addition to this, DNA sequence analysis allows obtainment of information about all nucleotides of the chosen genome fragment and the data of new mutations do not require improvement of the methods. The difficulty of results interpretation was simplified by means of the so-called "virtual phenotyping" that is based on comparison of information about the nucleotide sequence of the virus obtained from the patient with the international database of genotypes and phenotypes, which allows quantitative evaluation of the degree of sensitivity of the virus to a certain drug.

Risk of AIDS development before development of highly active antiretroviral therapy (HAART)

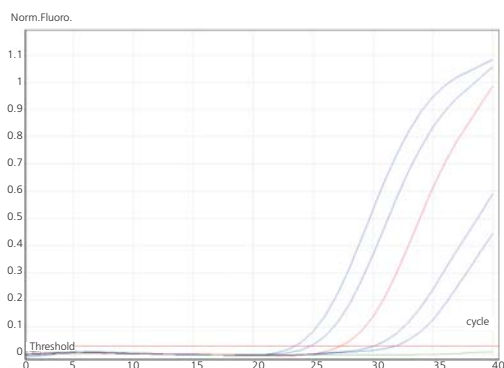


Risk of AIDS development at the HAART age

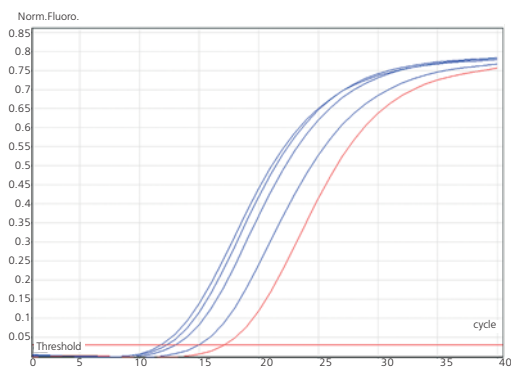


Reagent kits for detection of HIV provirus DNA in blood leukocytes

Representative works. FRT format



Joe/Yellow/Hex channel – HIV DNA



Fam/Green channel – endogenous internal control (human DNA)

Advantages of reagent kits

- Presence of a non-competitive internal control that allows prevention of falsely negative results, connected with non-effective isolation of cell DNA. Primers and probes of internal control are complementary to b-globin gene present in the human genomic DNA that is extracted from blood leukocytes with HIV provirus DNA.
- Application of primers and probes in the most conservative area of the HIV-1 polymerase gene that allow effective detection of the majority of HIV-1 subtypes, which was experimentally proved at the commercial panel of blood plasma samples containing 10 HIV-1 subtypes (NIBSC, cat. No.01/466).

Clinical material for examination

Clinical material	Recommended kits for extraction
Whole blood	→ 📄 Cytolis (1)
	→ 📄 Hem-sorb (2)

→ 📄 – a kit is included in the complete set reagent kit (📄)



FRT format - Fluorescence Detection in Real-Time Regime

Cat.No.	Name	Set.	No. of tests	Type	Mark	Special equipment
TR-V0-G (RG, IQ)	AmpliSens® DNA-HIV-FRT	📄 (2)	96	⚡	FAM/Green, JOE/HEX/ Yellow	Rotor-Gene 3000/6000 (Corbett Research), iCycler/iQ5 (BioRad)

Analytical properties

Sensitivity	10 GE per PCR-assay
Specificity	No cross reactions for viruses of hepatitis A, B, C, Delta, G and E, virus of chickenpox, Epstein-Barr virus, cytomegalovirus; virus of herpes simplex, types 1 and 2; human herpes virus, types 6, 7, 8; HPV virus, types 6, 11, 16, 18, 33, 35, as well as enteric virus strains (Coxsackie B1, B2, B3, B4, B5, B6, Polio I, II, III), respiratory viruses (adenoviruses of serogroups 5 and 7; viruses of A type flu), human rotavirus WA, astroviruses, noroviruses of types I and II.

Advantages of the format

- Detection of amplification products is carried out in the real-time regime (real-time PCR) without opening of test tubes, which reduces a risk of contamination to a significant degree
- A reagent kit is adapted for units Rotor-Gene (produced by Corbett Research, Australia) and iCycler (produced by BioRad, USA).

Results of clinical tests

Sensitivity and specificity of a reagent kit was determined in the process of state testing in L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute. The experimental group included 30 blood samples from HIV-infected patients at various stages of the disease. The control group included 15 blood samples of donors without antibodies to HIV, 15 blood samples of persons with various types of hepatitis, chronic infections (herpes, CMV) and pregnant women without antibodies to HIV. The specificity of the reagent kits made 100 percent: the result on

all samples from the control group was negative.

The sensitivity of reagents was assessed at examination of 30 blood samples from HIV-infected patients. From 30 samples positive result was obtained in all cases, which made 100 percent.

The “AmpliSens® DNA-HIV-FRT” complete set reagent kit is registered in the RF (registration certificate No. FS 01032006/4449-06 dated December 13, 2006).

HELISA format. Hybridization-Enzyme Linked ImmunoSorbent Analysis

Cat.No.	Name	Set.	No. of tests	Type	Special equipment
TH-1-G-R0,5	AmpliSens® DNA-HIV-96 M	(1)	96		Microtray spectrophotometer, microtray thermostat, voshier
TH-1-G-R0,2	AmpliSens® DNA-HIV-96 M	(1)	96		

Analytical properties

Sensitivity	10 GE per PCR-assay
Specificity	No cross reactions for viruses of hepatitis A, B, C, Delta, G and E, virus of chickenpox, Epstein-Barr virus, cytomegalovirus; virus of herpes simplex, types 1 and 2; human herpes virus, types 6, 7, 8; HPV virus, types 6, 11, 16, 18, 33, 35, as well as enteric virus strains (Coxsackie B1, B2, B3, B4, B5, B6, Polio I, II, III), respiratory viruses (adenoviruses of serogroups 5 and 7; viruses of A type flu), human rotavirus WA, astroviruses, noroviruses of types I and II.

HELISA format peculiarities

Detection of amplification products is carried out by means of hybridization –enzyme linked immunosorbent assay. The kit includes two microtrays: one – for analysis of HIV amplicon and the other –for ICS amplicon (a fragment of the globin gene).

Results of clinical tests

Sensitivity and specificity of a reagent kit was determined in the process of state testing in L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute. The experimental group included 60 blood samples from HIV-infected patients at various stages of the disease from IIB to IIIC, 17 blood samples from children born from HIV-infected mothers at the age younger than 6 months. The control group included 30 blood samples of donors without antibodies to HIV, 70 blood samples of persons with various types of hepatitis, chronic infections (herpes, CMV) and pregnant women without antibodies to HIV.

The specificity of the reagent kits made 100 percent: the result on all samples from the control group was negative.

The sensitivity of reagents was assessed at examination of 60 blood samples from HIV-infected patients. From 60 samples positive result was obtained in all cases, which made 100 percent.

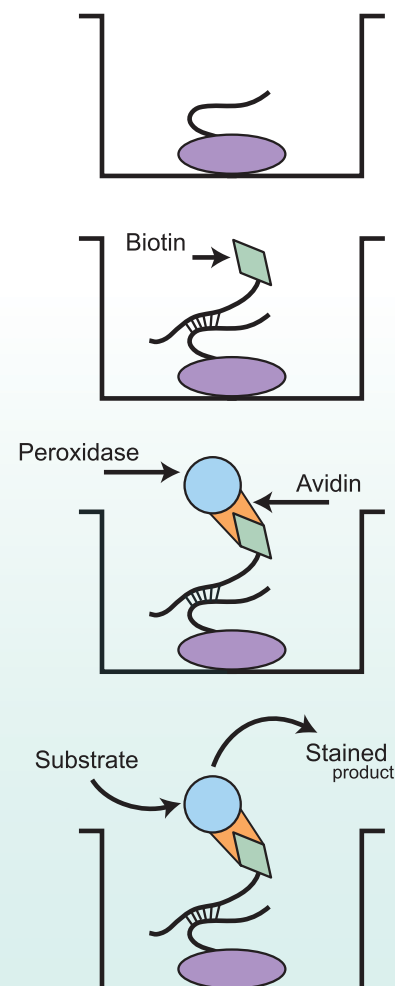
In the course of testing of blood samples from children born from HIV-infected mothers (17 children of various ages), 10 children with positive PCR results were detected. In the rest children positive results of the ELISA test and negative results of PCR analysis signify the carrier state of mothers' antibodies.

The “AmpliSens® DNA-HIV-96M” complete set reagent kit is registered in the RF (registration certificate No. L 001469 dated 31.03.2006).

Effectiveness of HIV subtypes detection in PCR with primers included in reagent kits AmpliSens® DNA-HIV-96 M, AmpliSens® HIV Monitor, AmpliSens® DNA-HIV-FRT and AmpliSens® HIV-Monitor-FRT

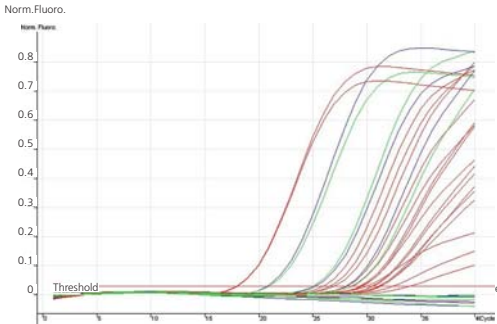
No.	Subtype	Roche	AmpliSens
1	C	3,68x10 ³	1,93x10 ⁴
2	H	1,25x10 ⁴	2,69x10 ⁴
3	A	4,31x10 ³	8,80x10 ³
4	N	2,63x10 ³	2,23x10 ³
5	B	2,26x10 ³	6,17x10 ³
6	E	5,05x10 ³	9,61x10 ³
7	O	-	-
8	D	2,81x10 ³	1,69x10 ⁴
9	G	4,82x10 ³	1,48x10 ³
10	F	5,08x10 ³	4,33x10 ³

Scheme of hybridization-enzyme linked immunosorbent analysis in cavities of the microtray

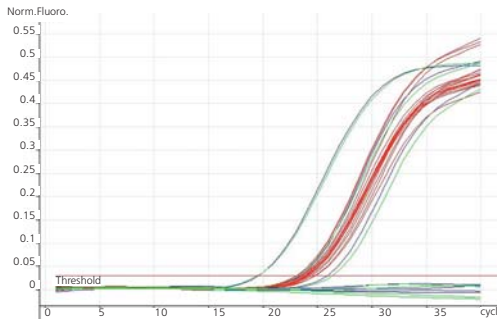


Reagent kits for quantitative determination of HIV RNA in blood plasma

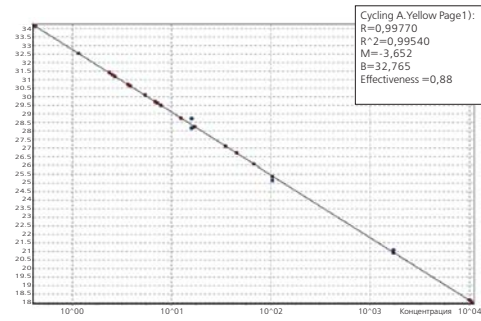
Representative works. Формат FRT



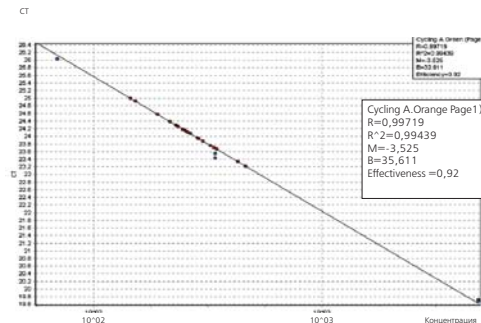
Joe/Yellow channel – HIV RNA



FAM/Green channel – quantitatively described internal control



Calibration straight line (Joe/Yellow channel – HIV RNA)



Calibration straight line (FAM/Green channel – internal control)

Advantages of reagent kits

- Application of primers and probes in the most conservative area of the HIV-1 polymerase gene that allow effective detection of the majority of HIV-1 subtypes
- Isolated RNA reverse transcription and cDNA amplification reactions are conducted in one reaction buffer with the help of the reverse transcriptase and Taq-polymerase.
- Use of the internal control sample introduced at the isolation stage that is quantitatively described and with the help of which HIV RNA concentration is calculated.
- Presence of two positive control samples that are quantitatively described and allow quality control of the conducted examinations.

Clinical material for examination

Clinical material	Recommended kits for extraction
Blood plasma	➔ RIBO-sorb, reagents NucliSENS for easyMAG

➔ — a kit is included in the complete set reagent kit ()

FRT format - Fluorescence Detection in Real-Time Regime

Cat.No.	Name	Set.	No. of tests	Type	Mark	Special equipment
TR-V0-M (RG,iQ, Mx)	AmpliSens® HIV-Monitor-FRT		48		FAM/Green, JOE/HEX/ Yellow	Rotor-Gene 3000/6000 (Corbett Research), iCycler/iQ5 (BioRad), Mx3000P (Stratagene)
R-V0-M (RG,iQ, Mx)	AmpliSens® HIV-Monitor-FRT		76			For isolation – an automatic unit NucliSENS for easyMAG is used

Advantages of format

- Detection of amplification products is carried out in the real-time regime (real-time PCR) without opening of test tubes, which reduces a risk of contamination to a significant degree.
- The reagent kit possesses a wider linear range of measurements.
- The reagent kits is adapted for units Rotor-Gene (produced by Corbett Research, Australia), iCycler, Q5 (produced by BioRad, USA) and Mx3000P (produced by Stratagene, USA).
- The reagent kit is adapted for automatic RNA extraction with the help of easyMAG unit (produced by BioMerieux, France).

Analytical properties

Sensitivity	500 copies per ml, a linear range of measurements: 500 – 5x10 ⁶ copies per ml; at use of easyMAG station: 50 copies per ml, a linear range of measurements: 50 – 5x10 ⁶ copies per ml.
Specificity	No cross reactions for viruses of hepatitis A, B, C, Delta, G and E, virus of chickenpox, Epstein-Barr virus, cytomegalovirus; virus of herpes simplex, types 1 and 2; human herpes virus, types 6, 7, 8; HPV virus, types 6, 11, 16, 18, 33, 35, as well as enteric virus strains (Coxsackie B1, B2, B3, B4, B5, B6, Polio I, II, III), respiratory viruses (adenoviruses of serogroups 5 and 7; viruses of A type flu), human rotavirus WA, astroviruses, noroviruses of types I and II.

Results of clinical tests

Sensitivity and specificity of the test as well as the linear range of measurements were determined in the process of state testing in L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute as compared to the

AmpliSens® HIV Monitor reagent kit. The experimental group included 36 plasma samples from HIV-infected patients at various stages of the disease, 16 plasma samples from 8 patients receiving antiviral therapy.

The analytical sensitivity is determined in copies of RNA virus per ml of plasma and makes 500 copies per ml. To increase analytical sensitivity of the kit to 50 copies per ml it's necessary to ultra-centrifuge 1 ml of blood plasma at 24.000 g (and more) for an hour in order to concentrate the virus in the examined sample or to conduct RNA extraction from 1 ml of blood plasma by an easyMAG unit.

The specificity of the reagent kit was evaluated on 20 samples of blood plasma obtained from donors without antibodies to HIV. The specificity made 100 percent.

The coefficient of variation was assessed by means of three fold examination of three clinical blood plasma samples. The

coefficient of variation made 29 percent.

Correlation of results with "AmpliSens® HIV Monitor" reagent kit

Comparison of results obtained by reagent kits "AmpliSens® HIV Monitor" and "AmpliSens® HIV Monitor-FRT" showed a high degree of concurrency: the coefficient of variation made 0.9; $p_v < 0,001$. Discordant results (when the difference between values exceeded 0.5 lg) were observed in 10 percent of cases.

The "AmpliSens® HIV Monitor-FRT" reagent kit is registered in the RF (registration certificate No. FS 01032006/5558-06 dated December 26, 2006).

HELISA format. Hybridization-Enzyme Linked ImmunoSorbent Analysis

Cat.No.	Name	Set.	No. of tests	Type	Special equipment
TH-1-M	AmpliSens® HIV Monitor		12		Microtray spectrophotometer, microtray thermostat, voshor

Analytical properties

Sensitivity	Sensitivity: 500 copies per ml Linear range of measurements: 500 – 800 000 copies per ml
Specificity	Samples containing the following microorganisms: viruses of B, C and D hepatitis, Epstein-Barr virus, viruses of herpes simplex of type 1 and 2, cytomegalovirus, papillomaviruses of types 11, 18, 16, 6, adenovirus, Chlamydia trachomatis, Chlamydia psittaci, Coxiela burnetti, Rickettsia prowazekii

Results of clinical tests

Sensitivity and specificity of the test as well as the linear range of measurements were determined in the process of state testing in L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute as compared to the "Cobas Amplicor HIV-1 Monitor" test system produced by Hoffman La Roche. The experimental group included 72 plasma samples from HIV-infected patients at various stages of the disease: from them - 5 samples from infected babies younger than 1 year, 30 plasma samples from 15 patients receiving antiviral therapy. In addition to this, the HIV international standard and its dissolution, a standard sample of the company and its dissolution as well as a commercial panel of subtypes were analyzed.

The analytical sensitivity is determined in copies of RNA virus per ml of plasma and makes 500 copies per ml. To increase analytical sensitivity of the kit to 50 copies per ml it's necessary to ultra-centrifuge 1 ml of blood plasma at 24.000 g (and more) for an hour in order to concentrate the virus in the examined sample or to conduct RNA extraction from 1 ml of blood plasma by an easyMAG unit.

The specificity of the reagent kit was evaluated on 106 samples of blood plasma obtained from donors without antibodies to HIV. The specificity made 100 percent.

The coefficient of variation was assessed by means of 10- fold examination of three clinical blood plasma samples. The coefficient of variation varied from 20 to 30 percent.

Correlation of results with the test system "Cobas Amplicor HIV-1 Monitor". Comparison of results obtained by reagent kits "AmpliSens® HIV Monitor" and "Cobas Amplicor HIV-1 Monitor" test system showed a high degree of concurrency: the coefficient of variation made 0.93; $p_v < 0,001$. At the average the difference between the values obtained by two reagent kits made 0.25 lg. Discordant results (when the difference between values exceeded 0.5 lg) were observed in 10 percent of cases. But in all cases the differences between the results didn't exceed 0.81 lg.

Detection of HIV-1 subtypes. The "AmpliSens® HIV Monitor" reagent kit detects with equal efficiency the majority of HIV-1 subtypes, which was proved by testing of the commercial panel of subtypes provided by the National Institute of Biological Standards and Controls of GB (NIBSC) (Cat.No. 01/466). The following HIV-1 subtypes were included in the panel: A, B, C, D, E, F, G, H, groups O and N. The reagent kit detected with a high effectiveness degree all subtypes of group M HIV-1, group N and didn't detect group O.

Adapted for the easyMAG unit (BioMerieux)



Representative works. HELISA format

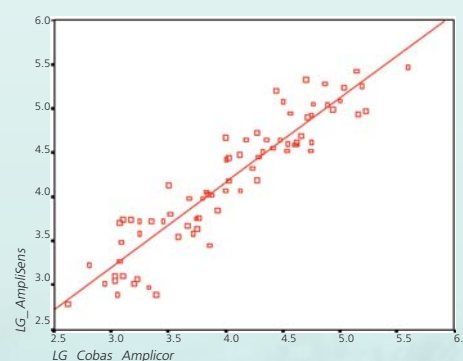
Results obtained in the process of the quantitative analysis

	AH 99/2.	AH 132/1	AK 3/1	AK 15/1	AK 16/1	AK 19/1	AK 59/1	AK 60/1	B -	L+	H+	B
1	2	3	4	5	6	7	8	9	10	11	12	
A	4.897	4.553	3.357	4.719	4.664	5.092	4.838	4.588	0.093	3.678	4.897	4.649
B	4.409	4.471	0.872	3.884	6.000	5.028	4.426	2.900	0.097	1.138	4.610	4.581
C	2.931	3.000	0.263	1.768	4.662	4.305	3.857	0.803	0.095	0.307	3.994	3.722
D	0.894	0.822	0.112	0.487	4.382	2.615	1.710	0.235	0.084	0.137	2.354	1.715
E	0.250	0.237	0.098	0.168	3.172	0.733	0.459	0.109	0.087	0.087	0.734	0.475
F	0.149	0.118	0.091	0.100	1.048	0.226	0.156	0.091	0.082	0.088	0.242	0.179
G	3.085	2.356	3.102	3.197	3.444	3.331	3.270	1.972	2.863	1.628	2.554	3.245
H	0.765	0.518	0.626	0.632	0.732	0.604	0.596	0.449	0.548	0.403	0.529	0.649

RNA concentration = (0.894 x 125) x 0.765 = 443 copies per ml

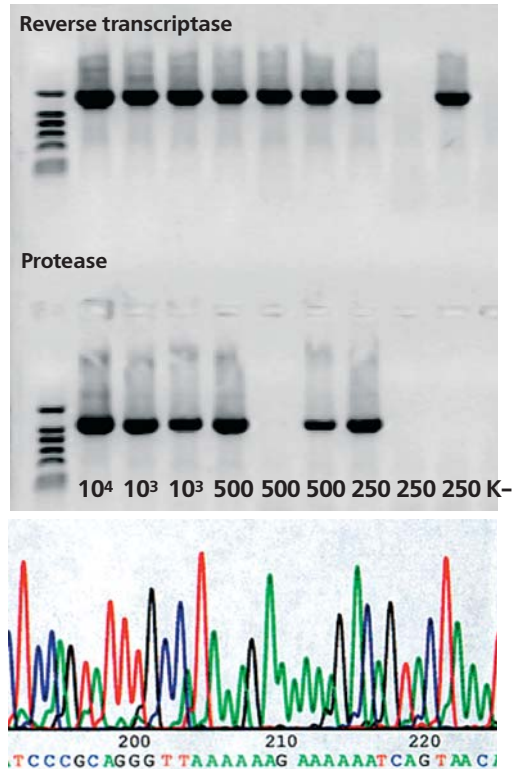
Correlation of results of viral charge measurement by "Cobas Amplicor HIV-1 Monitor v. 1.5" test system and "AmpliSens® HIV Monitor" reagent kit.

"AmpliSens® HIV Monitor" reagent kit is registered in the RF (registration certificate No. PN 003723/01 dated 20.08.2004)



Reagent kits for detection of mutations of HIV resistance to antiretroviral drugs

Representative works



In order to assess the analytical sensitivity of the reagent kit standard samples of the company (SSC) were developed containing HIV of A, B and G subtypes. Concentration of HIV RNA in samples of SSC was determined by the “Cobas Amplicor HIV-1 Monitor” test-system and the “AmpliSens® HIV Monitor” reagent kit. Specific products of amplification were obtained for all SSC irrespective of HIV subtype with concentration of more than 1,000 copies per ml.

Explanation:

Configuration of kits:

- a “complete set reagent kit» includes reagents for extraction, amplification and detection;

- an “amplification reagent kit” (PCR-set) includes only amplification reagents.

- a “reverse transcription and amplification reagent kit”

Kit types:

“Hot Start” is provided by a wax layer:

- a set includes ready to use PCR test tubes with the lower mixture applied under the wax

- a set includes vials with reagents not dispensed into PCR-test tubes.

“Hot Start” is provided by a modified polymerase (TaqF) activated at heating:

- a set includes vials with reagents not dispensed into PCR test tubes, a modified polymerase TaqF is used

Advantages of reagent kits

- A method of DNA sequence analysis is used to detect mutations of HIV resistance that allows obtainment of complete information about all nucleotide replacements in the chosen genome fragment.
- 2 HIV fragments are analyzed: a protease gene and a fragment of the reverse transcriptase gene (from 30 to 280 triplet)
- 3 primers are used for DNA sequence analysis, one of which - for protease gene and the other - for the reverse transcriptase gene.
- Reagents and equipments of different producers might be used for DNA sequence analysis.
- Interpretation of DNA sequence analyses is recommended to be carried out by the software in free access on site of Stanford University Hospital — <http://hivdb.stanford.edu> that allows conducting «virtual phenotyping».

Clinical material for examination и наборы реагентов

Clinical material	Recommended kits for extraction
Blood plasma	RIBO-sorb

— a kit is included in the complete set reagent kit (

Seq format – Detection by Sequence Analysis Method

Cat. No.	Name	Set.	No. of tests	Type	Special equipment
TM-V0-R0,2*	AmpliSens® HIV-genotype EPh		48		Electrophoretic chamber, sequenator, for example, Beckman Coulter

* — реагенты для реакции секвенирования не входят в состав набора

Analytical properties

Sensitivity	1,000 copies per ml
Specificity	No cross reactions for viruses of hepatitis A, B, C, Delta, G and E, virus of chickenpox, Epstein-Barr virus, cytomegalovirus; virus of herpes simplex, types 1 and 2; human herpes virus, types 6, 7, 8; HPV virus, types 6, 11, 16, 18, 33, 35, as well as enteric virus strains (Coxsackie B1, B2, B3, B4, B5, B6, Polio I, II, III), respiratory viruses (adenoviruses of serogroups 5 and 7; viruses of A type flu), human rotavirus WA, astroviruses, noroviruses of types I and II.

Results of clinical tests

Diagnostic sensitivity and specificity of the reagent kit was determined in the process of state testing in L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute.

The testing included laboratory control of three series of reagent kits and medical tests in two AIDS centers (a federal one and a regional center, Novosibirsk). The control group included 18 blood plasma samples from donors with negative reaction to HIV antibodies by results of ELIZA (10 samples from Moscow region and 8 – from the Novosibirsk region). The experimental group included 69 of blood plasma samples from HIV-infected patients: 26 patients – from Novosibirsk AIDS center, 27 – from Moscow region, 16 samples – from recently infected patients in Ulyanovsk who had never received any therapy. From 27 HIV-infected patients of the Moscow region 18 received combined antiretroviral therapy.

From 26 patients of the Novosibirsk region 17 samples were obtained from patients who had been on antiretroviral therapy less than a year ago.

At examination of blood plasma samples from HIV-infected patients for all samples with concentration of HIV-1 RNA over 1,000 copies per ml virus-specific fragments were obtained and analyzed. From 25 samples from patients who didn't received antiretroviral therapy 2 samples showed mutations causing HIV resistance to drugs. In the rest of the samples only secondary mutations or mutations of polymorphism characteristic of strains of subtype A HIV commonly spread in the territory of Russia.

From 33 patients who received combined therapy in 27 mutations causing drug resistance were observed (82 percent). The reverse transcriptase gene showed more than ten mutations causing resistance to drugs of various degrees.



Reagent kits for detection of HIV RNS as well as HCV RNA and HBV DNA in blood plasma

Advantages of reagent kits







- Presence of non-competitive internal control sample that is used through all stages of analysis starting from DNA isolation and prevents falsely negative results related to ineffective isolation of RNA.
- Reactions of reverse transcription of the isolated RNA and amplification of cDNA are conducted in a single reaction buffer with the help of the reverse transcriptase MMLV and Taq-polymerase.
- Detection of amplification products is conducted in real-time regime (Real-Time PCR) without opening of test tubes, which considerably reduces the risk of contamination.
- The reagent kit is adapted to units Rotor-Gene (Corbett Research, Australia) and iCycler/iQ5 (BioRad, USA).
- The reagent kit is adapted for automatic extraction of RNA by an easyMAG unit.

Clinical material for examination

Clinical material	Recommended kits for extraction
Blood plasma	➔ RIBO-sorb, reagents NucliSENS for easyMAG

➔  — a kit is included in the complete set reagent kit ()

FRT format - Fluorescence Detection in Real-Time Regime

Cat.No.	Name	Set.	No. of tests	Type	Mark	Special equipment
R-V0-R(RG,iQ)	AmpliSens® RNA-HIV-FL		48		FAM/Green, JOE/HEX/ Yellow	Rotor-Gene 3000/6000 (Corbett Research), iCycler/iQ5 (BioRad)
R-V50-4x(RG,iQ)	AmpliSens® HCV/ HBV/HIV-FL**		48		FAM/Green, JOE/HEX/ Yellow ROX/ Orange Cy5/Red	EasyMAG (BioMerieux), Rotor-Gene 3000/6000 (Corbett Research), iCycler/iQ5 (BioRad)
TR-V50-4x(RG,iQ)	AmpliSens® HCV/ HBV/HIV-FL**		48		FAM/Green, JOE/HEX/ Yellow ROX/ Orange Cy5/Red	Rotor-Gene 3000/6000 (Corbett Research), iCycler/iQ5 (BioRad)

** — reagent kits of MultiPrime series for simultaneous detection of HCV and HIV RNA and HBV DNA for provision of viral safety of donor blood

Analytical properties

Sensitivity	300 copies per ml, 50 copies per ml at use of ultra-centrifuging or easyMAG unit (BioMerieux)
Specificity	No cross reactions for viruses of hepatitis A, B, C, Delta, G and E, virus of chickenpox, Epstein-Barr virus, cytomegalovirus; virus of herpex simplex, types 1 and 2; human herpes virus, types 6, 7, 8; HPV virus, types 6, 11, 16, 18, 33, 35, as well as enteric virus strains (Coxsackie B1, B2, B3, B4, B5, B6, Polio I, II, III), respiratory viruses (adenoviruses of serogroups 5 and 7; viruses of A type flu), human rotavirus WA, astroviruses, noroviruses of types I and II.

Results of clinical tests

Analytical sensitivity. The standard method of RNA isolation from 100 µl of blood plasma allows reaching the sensitivity of 300 RNA per ml of plasma. To increase the test sensitivity ultra-centrifuging of a large amount of blood plasma (regime of centrifuging is more than 24,000 g, 1 hour at 2-8°C) might be used.

Specificity of the reagent kit made 100 percent: all samples of blood plasma obtained from donors produced negative result



AmpliSens® HCV/HBV/HIV-FL reagent kit

1. The kit is intended for simultaneous highly sensitive detection of hepatitis C and HIV viruses RNA and hepatitis B virus DNA (for screening of donor blood).
2. The kit is intended for automatic extraction of RNA/DNA with the easyMAG unit.
3. Detection in the real-time regime (FRT) on Rotor-Gene and iQ5 units.
4. A possibility to work with mini pools (up to 10 samples) without loss of analytical sensitivity.

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6. Pokrovskiy V.I. et al. Use of molecular-biological tests for screening of donor blood. Russian experience. // Healthcare and medical equipment – 2004. No. 8(12).
7. Bogoslovskaya E.V. et al. Test results of the Russian PCR-test-system «AmpliSens® HIV Monitor» for quantitative determination of HIV RNA in patients' blood plasma. // Biopreparations, 2005. No.2(18).
8. Zaitzev V.S. et al. Development of the «AmpliSens® DNA-HIV-FRT» test system on the basis of the real-time PCR method for detection of HIV DNA in whole blood. // Genodiagnostics of infectious diseases. Collected papers. Novosibirsk, 2005.
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12. Bogoslovskaya E.V. et al. Development and clinical testing of the test-system for quantitative determination of HIV RNA based on Real-Time PCR. // "Molecular diagnostics – 2007". Collected papers. Moscow, 2007.



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