AmpliSens® Genoscreen HLA B*5701-FRT PCR kit

RUO

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Contains sufficient for <n> tests
RUO	Research Use Only		Use-by Date
VER	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer	NCA	Negative control of amplification
	Date of manufacture	C–	Negative control of extraction
IC	Internal control	C+	Positive control of amplification

1. INTENDED USE

AmpliSens® Genoscreen HLA B*5701-FRT PCR kit is an *in vitro* nucleic acid amplification test for the detection of B locus 5701 allele of human major histocompatibility complex (HLA B*5701) in the biological material (whole blood and oropharyngeal swabs) using real-time hybridization-fluorescence detection of amplified products. The material for PCR is DNA samples extracted from test material.

Positive test result will be obtained if HLA B*5701 allele is presented either in homo- or heterozygous state. The probability of discrimination between the homo- and heterozygous alleles is absent when using the PCR kit. Positive test result can be obtained in the presence of related rare alleles: B*5514, B*5706, B*5708, B*5710, B*5713-B*5716, B*5718, B*5719 and B*5814 (less than 0.1 %).

Indications and contra-indications for use of the reagent kit

The reagent kit is used for the analysis of biological material taken from persons with confirmed *HIV* infection.

There are no contra-indications with the exception of cases when the material cannot be taken for medical reasons.

NOTE: For research use only. Not for diagnostic procedures.

2. PRINCIPLE OF PCR DETECTION

Principle of testing is based on the total human DNA extraction from the test material samples and simultaneous amplification of B locus region of major histocompatibility complex and DNA of the human β-globin gene with hybridization-fluorescence detection. DNA of the β-globin gene is used as an endogenous internal control (IC Glob) and allows not only to control all stages of the PCR study for each sample, but also to evaluate the adequacy of the material sampling, handling, transportation and storage.

Amplification of DNA fragments with the use of specific primers and Taq-polymerase enzyme are performed with the DNA samples obtained at the extraction stage. In the real-time PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes, which bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.

AmpliSens® Genoscreen HLA B*5701-FRT PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

The results of amplification are registered in the following fluorescence channels:

Table 1

Channel for fluorophore	FAM	JOE
DNA-target	β-globin gene region DNA (IC Glob)	B locus 5701 allele DNA
Target gene	β-globin gene	B locus 5701 allele

3. CONTENT

AmpliSens® Genoscreen HLA B*5701-FRT PCR kit is produced in 2 forms:

variant FRT REF R-O2(RG,iQ)-CE;

variant FRT in bulk[1] REF R-O2(RG,iQ)-CE-B.

Variant FRT includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FRT HLA	clear liquid from colorless to light lilac colour	0.6	2 tubes
RT-PCR-mix-2-FL	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	2 tubes
TE-buffer	colorless clear liquid	0.2	1 tube
Positive Control DNA HLA B*5701 and human DNA (C+$_{HLA\ B*5701}$)	colorless clear liquid	0.2	1 tube
Negative Control (C–)*	colorless clear liquid	0.5	4 tubes

* must be used in the extraction procedure as Negative Control of Extraction.

Variant FRT is intended for 110 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

For sampling and pretreatment

- Transport medium for storage and transportation of respiratory swabs.
- Reagent for pretreatment of whole blood.
- Vacuum tubes for sampling, storage and transportation of blood samples.
- Sterile swab with viscose tip in individual package.
- Disposable tightly closed polypropylene 1.5 or 2.0-ml tubes.
- Vacuum aspirator with flask for removing supernatant.
- Pipettes (adjustable).
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir to throw off and inactivate the material.
- Disposable powder-free gloves and a laboratory coat.

For DNA extraction and amplification

- DNA extraction kit.
- Sterile pipette tips with aerosol filters (up to 10 µl, 200 µl and 1000 µl).
- Tube racks.
- Vortex mixer.
- Thermostat with working temperature for 25-100 °C.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- Vacuum aspirator with flask for removing supernatant.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany); iCycler iQ or iCycler iQ5 (Bio-Rad, USA)).
- Disposable polypropylene tubes:
  a) 1.5-ml tubes – for DNA extraction;
  b) screwed or tightly closed 1.5-ml tubes for reaction mixture preparation;
  c) thin-walled 0.2-ml PCR tubes with optical transparent domed or flat caps or strips of eight 0.2-ml tubes with optical transparent caps if a plate-type instrument is used;
  d) thin-walled 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- Pipettes (adjustable).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.
- Disposable powder-free gloves and a laboratory coat.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use the PCR kit if the internal packaging was damaged or its appearance was changed.
- Do not use the PCR kit if the transportation and storage conditions according to the Instruction Manual were not observed.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.

[1] In bulk form contains unlabeled tubes. Tubes with identical reagent are packed in one bag with label.

- Avoid inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- Safety Data Sheets (SDS) are available on request.
- The PCR kit is intended for single use for PCR analysis of specified number of samples (see the section "Content").
- The PCR kit is ready for use in accordance with the Instruction Manual. Use the PCR kit strictly for intended purpose.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where the previous step was performed.

Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

6. SAMPLING AND HANDLING

AmpliSens® Genoscreen HLA B*5701-FRT PCR kit is intended for the analysis of the DNA extracted with DNA extraction kits from the biological material (whole blood and oropharyngeal swabs).

Sampling

Whole blood. Blood should be taken in an amount of 2 ml into a disposable sterile tube with EDTA (K_2EDTA or K_3EDTA) solution as the anticoagulant. The closed tube with blood should be rotated several times for the thorough mixing with the anticoagulant.
Whole blood samples can be stored before the pretreatment:
– at the temperature from 18 to 25 °C – for 2 hours,
– at the temperature from 2 to 8 °C – for 3 days.
Freezing of venous whole blood samples is unacceptable!
Oropharyngeal swabs. Rotate the working part of the swab over the surface of tonsils, palatine arches, and posterior wall of pharynx. Transfer the swab into a tube with 500 µl of **Transport Medium for Storage and Transportation of Respiratory Swabs**. Break off the working part of the swab which contains test material and leave it in the tube with transport medium. Close the tube tightly excluding gap and deformation of the interior part of the cap. If it is impossible to break the swab, insert the working part of the swab into the transport medium and rotate it against the interior wall of the tube for 5-10 seconds, then remove the swab and close the tube tightly.
The using of scissors for cutting the working part of the swab is unacceptable!
Oropharyngeal swabs can be stored before the PCR-analysis:
− at the temperature from 18 to 25 °C – for 6 hours;
− at the temperature from 2 to 8 °C – for 3 days;
− at the temperature from minus 24 to minus 16 °C – for 2 weeks;
− at the temperature not more than minus 68 °C – for a long time.
Only one freeze-thawing cycle is acceptable.

Pretreatment

Pretreatment of oropharyngeal swabs is not required.
Whole blood samples are to be pretreated.
Collect 100 µl of *whole blood* into the disposable 1.5-ml tube. Add 1.0 ml of **Hemolytic**. Close the tubes, gently vortex and leave them for 5 min at the room temperature, then again gently vortex the tubes; leave them for 5 min. Centrifuge the tubes at 8,000 rpm for 2 min. Remove the supernatant without disturbing the pellet using a vacuum aspirator and a new tip for each tube. After washing the cell pellet should be white, only a small pinkish bloom on the pellet is allowed (the remains of the destroyed erythrocytes). The washing with **Hemolytic** may be repeated if necessary. The obtained pellet must be immediately lysed (in case of extraction using **RIBO-prep** add **300 µl** of **Solution for Lysis** and then extract DNA in accordance with the *Instruction Manual* enclosed to the RIBO-prep reagent kit without adding **Solution for Lysis** once again) or freezed.
The freezed pellet can be stored:
− at the temperature from minus 24 to minus 16 °C – for 2 weeks;
− at the temperature not more than minus 68 °C – for a long time.

Interfering substances and limitations of using test material samples

The next samples are inapplicable for analysis:
– venous whole blood samples collected in the tubes with heparin as anticoagulant,
– venous whole blood samples containing blood clot or which has been exposed to freezing.

In order to control the DNA extraction efficiency and amplification the DNA of β-globin gene is used as an endogenous internal control (IC Glob) in the PCR kit. The presence of internal control signal after the amplification testifies the effectiveness of nucleic acid extraction and the absence of PCR inhibitors.

Potential interfering substances

Endogenous and exogenous substances that may be present in the biological material used for the study were selected to assess potential interference (see Table 2).
Whole blood samples and oropharyngeal swabs without adding and with the addition of potential interfering substances were tested. The concentration of each potential interfering substance is specified in Table 2.
Whole blood samples and oropharyngeal swabs from patients with confirmed B locus 5701 allele of human major histocompatibility complex (HLA B*5701) were tested. Simultaneously, samples of biological material from persons with confirmed absence of the HLA B*5701 allele containing each endogenous potentially interfering substance and without adding these substances (negative control sample) were tested.

Table 2

Test material	Type of potential interferent	Potential interferent (tested concentration in a sample)	Interference presence
Whole blood	Endogenous substances	Hemoglobin (5 g/l)	Not detected
		Triglycerides (37 mmol/l)	Not detected
		Bilirubin (210 µmol/l)	Not detected
		Cholesterol (77.6 mmol/l)	Not detected
	Exogenous substances	K_2EDTA (2 mg/ml)	Not detected
		K_3EDTA (2 mg/ml)	Not detected
		Lithium heparin (12 IU/ml)	Detected
Oropharyngeal swabs	Endogenous substances	Mucin (6 mg/ml)	Not detected
		Mucin (9 mg/ml)	Not detected
		Hemoglobin (0.21 g/ml)	Not detected
	Exogenous substances	Chlorhexidine bigluconate aqueous solution (2.5 %)	Not detected

7. WORKING CONDITIONS

AmpliSens® Genoscreen HLA B*5701-FRT PCR kit should be used at the temperature from 20 to 28 °C and relative humidity from 15 to 75 %.

8. PROTOCOL

8.1. DNA extraction

It is recommended to use the following nucleic acid extraction kits:
- **RIBO-prep**.

In the extraction procedure it is necessary to carry out the control reaction as follows:

C– - Add **100 µl** of **Negative Control (C–)** to the tube labelled **C–** (Negative Control of Extraction).

Extract the DNA according to the manufacturer's protocol taking into account next additions and improvements:

NOTE:

When using leukocyte pellet obtained after the preparation of whole blood samples:
1. Add **300 µl** of **Solution for Lysis** to the each tube with leukocyte pellet.
2. Add **300 µl** of **Solution for Lysis** to the tube for Negative Control of Extraction (C–).

When using oropharyngeal swabs:
1. Take the required number of 1.5-ml disposable tubes (including Negative Control of Extraction (C–)). Mark the tubes.
2. Add **300 µl** of **Solution for Lysis** to the tubes.
3. Add **100 µl** of **test samples** to the tubes using a separate pipette tip with filter for each sample.

8.2. Preparing PCR

8.2.1 Preparing tubes for PCR

The total reaction volume is **25 µl**, the volume of DNA sample is **10 µl**.
Use disposable filter tips for adding reagents, DNA and control samples into tubes.
1. Calculate the required quantity of each reagent for reaction mixture preparation. For one reaction:
   – **10 µl** of **PCR-mix-1-FRT HLA**,
   – **5 µl** of **RT-PCR-mix-2-FL**,
   – **0.5 µl** of **Polymerase (TaqF)**.

Prepare the reaction mixture for the total number of test and control samples plus some extra reaction. See numbers of control samples in item 7.
The calculation for the required number of reactions including testing the test and control samples can be performed according to Table 3.

NOTE: Prepare the reaction mixture just before use.

2. Thaw all the reagents of the PCR kit, vortex thoroughly and sediment the drops by short centrifugation.
3. In a new tube prepare the reaction mixture. Mix the required quantities of **PCR-mix-1-FRT HLA, RT-PCR-mix-2-FL** and **Polymerase (TaqF)**. Vortex thoroughly and sediment the drops from the caps of the tubes by short centrifugation. The prepared mixture is not to be stored.

Table 3

Scheme of reaction mixture preparation

			Reagent volume for specified number of reactions plus extra reactions, µl		
Reagent volume per one reaction, µl			10.0	5.0	0.5
Number of test samples	Number of samples to be extracted[2]	Number of reactions[3]	PCR-mix-1-FRT HLA	RT-PCR-mix-2-FL	Polymerase (TaqF)
3	4	6	60	30	3.0
4	5	7	70	35	3.5
5	6	8	80	40	4.0
6	7	9	90	45	4.5
7	8	10	100	50	5.0
8	9	11	110	55	5.5
9	10	12	120	60	6.0
10	11	13	130	65	6.5
11	12[4]	14	140	70	7.0

4. Take the required number of the tubes or strips for DNA amplification according to the number of test and control samples (see Table 3).
5. Transfer **15 µl** of the prepared reaction mixture to each tube. Discard the unused reaction mixture.
6. Add **10 µl** of **DNA samples** obtained by extraction of the test samples.
7. Carry out the control reactions:

C+ – Add **10 µl** of **Positive Control DNA HLA B*5701 and human DNA (C+$_{HLA\ B*5701}$)** to the tube labeled C+ (Positive Control of Amplification).
C– – Add **10 µl** of **the sample extracted from the Negative Control (C–)** to the tube labeled C– (Negative Control of Extraction).
NCA – Add **10 µl** of **TE-buffer** to the tube labelled NCA (Negative Control of Amplification).

8.2.2. Amplification

1. Create a temperature profile on your instrument as follows:

Table 4

Amplification program for rotor-type instruments[5]

Step	Temperature, °C	Time	Fluorescent signal detection	Cycles
1	95	15 min	–	1
2	95	5 s	–	5
	60	20 s	–	
3	95	5 s	–	40
	60	40 s	FAM, JOE	

Table 5

Amplification program for plate-type instruments[6]

Step	Temperature, °C	Time	Fluorescent signal detection	Cycles
1	95	15 min	–	1
2	95	5 s	–	5
	60	20 s	–	
3	95	5 s	–	40
	60	50 s	FAM, JOE	

[2] Number of test samples plus one control of DNA extraction stage (N+1, N – number of test samples).
[3] Number of test samples plus one control of DNA extraction stage plus two controls of PCR (N+3, N – number of test samples).
[4] Panel of 12 test tubes for extraction.
[5] For example, Rotor-Gene 3000/6000 (Corbett Research, Australia), Rotor-Gene Q (QIAGEN, Germany).
[6] For example, iCycler iQ, iQ5 (Bio-Rad, USA).

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin*.
3. Insert tubes into the reaction module of the device.

NOTE: It is recommended to sediment drops from walls of tubes by short centrifugation (1–3 s) before placing them into the instrument.
Insert empty tubes at the edges of reaction module in case of incomplete filling of plate-type instrument.

4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in two channels:

Table 6

Channel for the fluorophore	FAM	JOE
Amplification product	β-globin gene region DNA (IC Glob)	B locus 5701 allele DNA

Results are interpreted by the crossing (or not-crossing) the S-shaped (sigmoid) fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the DNA sample in the corresponding column of the results grid.
Principle of results' interpretation is the following:

Table 7

Results interpretation for the test samples

***Ct* value in the channel for the fluorophore**		**Result**
FAM	**JOE**	
≤ boundary value	determined and does not exceed the *Ct* value in the FAM channel by more than 5 cycles	B locus 5701 allele **is detected**
≤ boundary value	absent or exceeds the *Ct* value in the FAM channel by more than 5 cycles	B locus 5701 allele **is NOT detected**
absent or > boundary value	determined or absent	**Equivocal**

NOTE: Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

The result of the analysis is considered reliable only if the results obtained for Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 8).

Table 8

Results for controls

Control	**Stage for control**	***Ct* value in the channel for fluorophore**	
		FAM	**JOE**
C–	DNA extraction	absent or > boundary value	absent
NCA	PCR	absent	absent
C+	PCR	< boundary value	< boundary value

10. TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:

1. The *Ct* value for the Positive Control of amplification (C+) is absent in any of the specified channels for fluorophores (see Table 8). The amplification and detection should be repeated for all the samples in which specific DNA was not detected.
2. The *Ct* value for the test sample is not determined in the channel for the FAM fluorophore. The PCR analysis (beginning with the DNA extraction stage) should be repeated for the appropriate test sample.
3. The *Ct* value determined for the test sample in the channel for the FAM fluorophore is greater than the boundary value. In this case the sample is considered **equivocal**. The PCR analysis (beginning with the DNA extraction stage) should be repeated for the appropriate test sample.
4. The *Ct* value is determined for the Negative Control of Extraction (C–) in the channel for the JOE fluorophore and/or for the Negative Control of amplification (NCA) in the channels for the FAM and/or JOE fluorophores. The contamination of laboratory with amplification products or contamination of reagents, test samples is probable at any stage of PCR analysis. Measures for detecting and elimination of contamination source must be taken. The amplification and detection should be repeated for all the samples in which specific DNA was detected.

11. TRANSPORTATION

AmpliSens® Genoscreen HLA B*5701-FRT PCR kit should be transported at 2–8 °C for no longer than 5 days. PCR kit can be transported at 2–25 °C for no longer than 3 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® Genoscreen HLA B*5701-FRT** PCR kit are to be stored at temperature from minus 24 to minus 16 ºC when not in use.
All components of the **AmpliSens® Genoscreen HLA B*5701-FRT** PCR kit are stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: PCR-mix-1-FRT HLA is to be kept away from light.

13. SPECIFICATIONS

13.1. Analytical sensitivity (limit of detection)

Table 9

Test material	The volume of sample for extraction, µl	Nucleic acid extraction kit	PCR kit	Analytical sensitivity (limit of detection), cells/ml
Whole blood, oropharyngeal swabs	100	RIBO-prep	variant FRT	$1x10^3$

The claimed limit of detection is achieved while respecting the rules specified in the section "*Sampling and Handling".*

13.2. Analytical specificity

The analytical specificity of **AmpliSens® Genoscreen HLA B*5701-FRT** PCR kit is ensured by the selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.
The analytical specificity of the PCR kit was proved when investigating the RNA/DNA of the following microorganisms/viruses at concentration no more than $1x10^7$ copies/ml (GE/ml) and no less than $1x10^3$ copies/ml (GE/ml): *Adenovirus* types 2, 3, 7, *Cytomegalovirus* (*CMV*), *Epstein-Barr virus* (*EBV*), *Varicella-zoster* virus (*VZV*), *Hepatitis A virus* (*HAV*), *Hepatitis B virus* (*HBV*), *Hepatitis C virus* (*HCV*), *Human immunodeficiency virus* type 1 (*HIV*-1), *Human herpes virus* types 6, 8 (*HHV*-6, *HHV*-8), *Herpes simplex virus* types 1, 2 (*HSV*-1,2), *Rubella virus*, *Human papillomavirus* types 6, 11, 16, 18, 31, 33, 39, 51, 52, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Treponema pallidum*.
The nonspecific reactions were absent while testing RNA/DNA samples of the above-mentioned microorganisms and viruses.
The analytical specificity of the reagent kit was assessed using a panel of 13 genetically engineered DNA constructs with different combinations of human HLA-B alleles provided by the Institute for Immunology and Infectious Diseases, Murdoch University, Australia.
The information about interfering substances is specified in the *Interfering substances and limitations of using test material samples*.

13.3. Reproducibility and repeatability

Repeatability and reproducibility were determined by testing of positive and negative model samples. Positive samples were the quality control sample (QCS) containing HLA B*5701 DNA at concentration of $1.3x10^6$ copies/ml. Negative control (C–) reagent was used as a negative sample.
Repeatability conditions included testing in the same laboratory, by the same operator, using the same equipment within a short period of time. Reproducibility conditions included testing different lots of PCR kit in different laboratories, by different operators, on different days, using different equipment. The results are presented in Table 10.

Table 10

Sample type	Repeatability		Reproducibility	
	Number of samples	Agreement of results, %	Number of samples	Agreement of results, %
Positive	10	100	40	100
Negative	10	100	40	100

14. REFERENCES

1. Cost-effectiveness analysis of HLA-B*5701 typing in the prevention of hypersensitivity to abacavir in HIV+ patients in Spain// Enferm Infecc Microbiol Clin. 2010 Nov;28(9):590-5. doi: 10.1016/j.eimc.2009.09.010.
2. Low prevalence of human leukocyte antigen-B*5701 in HIV-1-infected Chinese subjects: a prospective epidemiological investigation // AIDS Res Ther. 2015 Aug 19;12:28. doi: 10.1186/s12981-015-0064-9. eCollection 2015.
3. Low-cost simultaneous detection of CCR5-delta32 and HLA-B*5701 alleles in human immunodeficiency virus type 1 infected patients by selective multiplex endpoint PCR // J Virol Methods. 2015 Nov;224:102-4. doi: 10.1016/j.jviromet.2015.08.020. Epub 2015 Sep 2.
4. Validation of two commercial real-time PCR assays for rapid screening of the HLA-B*57:01 allele in the HIV clinical laboratory // J Virol Methods. 2016 Nov;237:18-24. doi: 10.1016/j.jviromet.2016.08.013. Epub 2016 Aug 18.

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science "Central Research Institute for Epidemiology" ISO 13485-Certified Quality Management System, each lot of the **AmpliSens® Genoscreen HLA B*5701-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
07.06.11 VV	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
	Catalogue number	R-O2(RG,iQ)-CE was deleted
	Content	Form AmpliSens® Genoscreen HLA B*5701-FRT PCR kit variant FRT (for use with RG, iQ) REF R-O2(RG,iQ)-CE was deleted
	Key to Symbols Used	The explanation of symbols was corrected
20.06.11 VV	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
05.04.13 PE	Cover page	The symbol IVD was changed to RUO
	Content	The name of positive control was changed from “Positive Control DNA HLA B*5701 and human DNA (C+)” to “Positive Control DNA HLA B*5701 and human DNA ($C+_{HLA\ B*5701}$)”
26.07.16 PM	Text	Corrections according to the template
	8. Protocol	Information about carrying out the Negative control of extraction was added
	9. Data analysis	The sections were rewritten. Information about result interpretation and troubleshooting for C– was corrected
	10. Troubleshooting	
	14. References	The references to guidelines was added
31.05.21 MM	3. Content	The color of the reagent was specified
	2. Principle of PCR detection	The table with targets was added
	Through the text	The text formatting was changed
07.09.23 EM	Through the text	Corrections according to the template
	1. Intended use	The subsection *Indications and contra-indications for use of the reagent kit* was added
	3. Content Footer	**REF** R-O2(RG,iQ)-CE was added
	3. Content	The volume and quantity of TE-buffer reagent were changed
	6. Sampling and handling	The information about sampling and handling was expanded. The subsection *Interfering substances and limitations of using test material samples* was added
	8. Protocol	The subsection *8.1.1 DNA extraction using RIBO-prep nucleic acid extraction kit* was added
	10. Troubleshooting	The section was rewritten
	13. Specifications	The subsection *13.3. Reproducibility and repeatability* was added
	14. References	The section was actualized

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