# AmpliSens® *JCV-BKV* screen/monitor-FRT PCR kit

IVD

For Professional Use Only

# Instruction Manual

## KEY TO SYMBOLS USED

| | | | |
|---|---|---|---|
| REF | Catalogue number | | Caution |
| LOT | Batch code | Σ | Contains sufficient for <n> tests |
| IVD | *In vitro* diagnostic medical device | | Use-by Date |
| VER | Version | | Consult instructions for use |
| | Temperature limit | | Negative control of amplification |
| | Manufacturer | C– | Negative control of extraction |
| | Date of manufacture | C+ | Positive control of Amplification |
| EC REP | Authorized representative in the European Community | C1, C2 | DNA-calibrators |
| PCE | Positive control of extraction | IC | Internal control |

## 1. INTENDED USE

**AmpliSens® *JCV-BKV* screen/monitor-FRT** PCR kit is an *in vitro* nucleic acid amplification test for quantitative detection of *JC virus (JCV)* DNA in the biological material (whole blood, cerebrospinal fluid (CSF)) and *BK virus (BKV)* DNA in the biological material (whole blood, urine) taken from the persons suspected of progressive multifocal leukoencephalopathy, *JC*-encephalopathy, meningitis, encephalomeningitis, encephalitis caused by *JC virus* and *BK virus* associated nephropathy without distinction of form and presence of manifestation, using real-time hybridization-fluorescence detection of amplified products.

**NOTE:** The results of PCR analysis are taken into account in complex diagnostics of disease.

## 2. PRINCIPLE OF PCR DETECTION

The principle of testing is based on the DNA extraction from test samples together with the exogenous internal control (Internal Control-FL (IC)) and simultaneous amplification of DNA fragments of the detected microorganism and DNA of the internal control with hybridization-fluorescence detection. Exogenous internal control (Internal Control-FL (IC)) allows to control all PCR-analysis stages of each individual sample and to identify possible reaction inhibition.

*JCV* and *BKV* detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific primers. 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.

The quantitative analysis of *JCV* and *BKV* DNA is based on the linear dependence between the initial concentration of DNA target in a test sample and the cycle threshold (*Ct*) (the cycle of beginning of fluorescence signal exponential growth). For the quantitative analysis amplification of DNA from the test samples is carried out simultaneously with DNA-calibrators (samples with the known concentration of the DNA target). Based on the amplification results of DNA-calibrators a calibration line is plotted and it is used for the estimation of concentration of the DNA target in the test samples.

At the amplification stage 3 reactions are carried out in one tube simultaneously: amplification of DNA fragments of *JCV* and *BKV* as well as amplification of Internal Control-FL (IC) DNA. The results of amplification of *JCV* and *BKV* DNA and Internal Control-FL (IC) DNA are registered in 3 different fluorescence channels.

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

Table 1

| Channel | FAM | JOE | ROX |
|---|---|---|---|
| DNA-target | Internal Control-FL (IC) DNA | *JCV* DNA | *BKV* DNA |
| Target gene | Artificially synthesized sequence | large T-antigen gene | large T-antigen gene |

## 3. CONTENT

**AmpliSens® *JCV-BKV* screen/monitor-FRT** PCR kit is produced in 1 form:

**variant FRT-100 F**, REF H-2441-1-1-CE.

**Variant FRT-100 F** includes:

| Reagent | Description | Volume, ml | Quantity |
|---|---|---|---|
| **PCR-mix-FL *JCV-BKV*** | clear liquid from colorless to light lilac colour | 1.2 | 1 tube |
| **PCR-buffer-B** | colorless clear liquid | 0.6 | 1 tube |
| **Polymerase (TaqF)** | colorless clear liquid | 0.06 | 1 tube |
| **C1 *JCV-BKV*** | colorless clear liquid | 0.2 | 1 tube |
| **C2 *JCV-BKV*** | colorless clear liquid | 0.2 | 1 tube |
| **TE-buffer** | colorless clear liquid | 0.2 | 1 tube |
| **Internal Control-FL (IC)*** | colorless clear liquid | 1.0 | 1 tube |
| **Negative Control (C-)**** | colorless clear liquid | 1.2 | 2 tubes |
| **Positive Control *JCV-BKV****** | colorless clear liquid | 0.1 | 1 tube |

* add **10 µl** of **Internal Control-FL (IC)** during the DNA extraction procedure directly to the sample/lysis mixture (see **RIBO-prep** protocol).

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

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

**Variant FRT-100 F** is intended for 110 reactions (including controls).

## 4. ADDITIONAL REQUIREMENTS

- Vacuette® blood collection system.
- Puncture needles.
- Disposable plastic container (50-60 ml) for sampling, storage and transportation of biological samples.
- Reagent for pretreatment of whole peripheral and umbilical blood.
- Vacuum aspirator with flask for removing supernatant.
- Vortex mixer.
- Vacuum aspirator with flask for removing supernatant.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- DNA extraction kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with filters (up to 100 µl, 200 µl and 1000 µl).
- Tube racks.
- PCR box.
- Real-time instruments with 3 (or more) independent detection channels (for example, Rotor-Gene Q (QIAGEN, Germany), CFX96 (Bio-Rad, USA)).
- Disposable polypropylene PCR tubes:
  a) tightly closed 2.0-ml tubes for sampling;
  b) screwed or tightly closed 1.5-ml tubes for pretreatment and 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.
- Refrigerator with the range from 2 to 8 °C.
- Deep-freezer with the range from minus 24 to minus 16 °C.
- Reservoir for used tips.

## 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 positive material (specimens, controls) away from all other reagents and add it to the reaction mix in a distantly separated facility. Store and handle amplicons away from all other reagents.
- 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 afterward.
- 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 samples and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance 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.
- Avoid samples and reagents contact with the skin, eyes and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- 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 the 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 to 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® *JCV-BKV* screen/monitor-FRT** PCR kit is intended for the analysis of DNA extracted with DNA extraction kits from the biological material (whole blood, cerebrospinal fluid (CSF), urine).

***Sampling***

6.1 *Whole blood.* Blood should be taken after overnight fasting or in 3 hour after eating by a disposable 0.8-1.1 mm diameter needle into the tube with EDTA (special vacuum system Vacuette® (lavender caps – 6 % EDTA)). After blood sampling the tube should be gently inverted several times for the thoroughly mixing with the anticoagulant. (Otherwise, blood will coagulate and DNA extraction will be impossible!)

The samples can be stored before the pretreatment:

— at the temperature from 18 to 25 °C – for 6 hours after material sampling,

— at the temperature from 2 to 8 °C – for 1 day.

6.2 *Cerebrospinal fluid (CSF).* Cerebrospinal fluid is collected in an amount no less than 1 ml by puncturing the lumbar, suboccipital area, or cerebral ventricles using sterile puncture needle into disposable 2.0-ml tubes.

The cerebrospinal fluid samples can be stored before the PCR analysis:

— at the temperature from 2 to 8 °C – for 1 week,

— at the temperature from minus 24 to minus 16 °C – for 1 month,

— at the temperature ≤ –68 °C – for a long time.

Only one freeze-thawing cycle is required

6.3 *Urine.* The first portion of first void urine is taken for PCR-analysis in an amount of 20-40 ml into the dry sterile container (50-60 ml).

The urine samples can be stored:

— at the temperature from 18 to 28 °– for 1 day;

— at the temperature from 2 to 8 °C – for 1 day;

— at the temperature from minus 24 to minus 16 °C – for 1 week;

— at the temperature ≤ –68 °C – for a long time

Only one freeze-thawing cycle is acceptable.

It is allowed to transport the above-mentioned material at the temperature from 2 to 8 °C for 1 day.

***Pretreatment***

6.4 *Cerebrospinal fluid (CSF).* Pretreatment is not required in case of extraction from 100 ml. It is allowed to concentrate the sample from a larger volume (for example, from 1 ml) To do this, centrifuge the sample at 10,000 g (for example, 12,000 rpm for the MiniSpin Eppendorf microcentrifuge) for 5 min. After centrifugation carefully remove the supernatant using the vacuum aspirator and leaving the pellet and 100 µl of supernatant.

**NOTE:** It is necessary to take into account the volume of the sample before pretreatment while calculating the concentration (see the section "Data analysis")

6.5 *Whole blood.* The whole blood samples are to be pretreated. Transfer 0.25 ml of whole blood to the disposable 1.5-ml tube. Add 1.0 ml of **Hemolytic**. Gently vortex the tubes and leave them for 10 minutes at room temperature (from 18 to 25°C), stirring occasionally. Centrifuge at 4,000 g (for example, 8,000 rpm for the MiniSpin Eppendorf microcentrifuge) for 2 minutes. Remove the supernatant using vacuum aspirator leaving 100 µl of the pellet. 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 using **Hemolytic** may be repeated if necessary. The obtained leucocytes 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).

The pretreated samples of whole blood can be stored before the PCR-analysis:

- at the temperature from minus 24 to minus 16 °C – for 1 year;

Only one freeze-thawing cycle is acceptable.

6.6. *The urine samples* are to be pretreated.

Shake the vial (container) with urine. Transfer 1 ml of urine into the sterile disposable 1.5-ml tube using filter tip. Centrifuge at 10,000 g (for example, 12,000 rpm for the MiniSpin Eppendorf microcentrifuge) for 5 min. Carefully remove the supernatant using the vacuum aspirator and leaving the pellet and 100 µl of supernatant.

**NOTE:** It is necessary to take into account the volume of the sample before pretreatment while calculating the concentration (see the section "Data analysis")

The pretreated urine samples can be stored before the PCR-analysis:

- at the temperature from 18 to 25 °C – for 1 day;
- at the temperature from 2 to 8 °C – for 1 day;
- at the temperature from minus 24 to minus 16 °C – for 2 months;
- at the temperature not more than 68 °C – for a long time.

Only one freeze-thawing cycle is acceptable.

It is allowed to transport the above-mentioned material at the temperature from 2 to 8 °C for 1 day.

***Interfering substances and limitations of using test material samples***

In order to control the DNA extraction efficiency and possible reaction inhibition the Internal Control (Internal Control-FL (IC)) is used in the PCR kit. The Internal Control is added in each biological sample at the extraction stage. The presence of internal control signal after the amplification testifies the effectiveness of nucleic acid extraction and the absence of PCR inhibitors.

The next samples are inapplicable for analysis:

- the urine samples collected more than 24 hours before delivery to the laboratory,
- the whole blood samples, collected in the tubes with heparin as anticoagulant,
- the whole blood samples, containing blood clot or which has been exposed to freezing.

## 7. WORKING CONDITIONS

**AmpliSens® *JCV-BKV* screen/monitor-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**.

**NOTE:**
**If using the RIBO-prep kit** extract the DNA according to the manufacturer's protocol.
The volumes of reagents and samples when the DNA is extracted by the RIBO-prep reagent kit:
The DNA extraction for each sample is carried out in the presence of **Internal Control-FL (IC).**
Add **10 µl** of **Internal Control-FL (IC)** to each tube.
The volume of the test sample is **100 µl.**
Add **100 µl of Negative Control (C–)** into the tube labeled C– (Negative Control of Extraction).
Add **10 µl of Positive Control *JCV-BKV*** and **90 µl of Negative Control (C–)** into the tube labeled PCE (Positive Control of Extraction).
The volume of elution is **50 µl.** It is allowed to increase the volume of elution to **90 µl** if it is necessary**.**

### 8.2. Preparing PCR

#### 8.2.1 Preparing tubes for PCR

The total reaction volume is **25 µl**, the volume of the DNA sample is **10 µl**.

The type of tubes depends on the PCR instrument used for analysis. Use disposable filter tips for adding reagents, DNA and control samples into tubes.

1. It is necessary to prepare the mixture of **PCR-buffer-B** and **polymerase (TaqF)**. Transfer the entire content of one tube with **polymerase (TaqF)** (60 µl) into the tube with **PCR-buffer-B** (600 µl). Carefully vortex the tube avoiding foaming and then centrifuge on vortex for 1-2 s. Avoid foaming. Mark the tube and indicate the date of mixture preparation.

**NOTE:** The prepared mixture is intended for analysis of 120 samples. The mixture should be stored at 2–8 °C for up to 3 months and used as necessary.

**NOTE:** **If the mixture cannot be utilized within 3 months, it should be prepared for a smaller number of reactions. For example, mix 150 µl of PCR-buffer-B and 15 µl of polymerase (TaqF). Thus, prepared mixture is intended for 30 reactions.**

2. Calculate the required quantity of each reagent for reaction mixture preparation. For one reaction:
   - **10 µl** of **PCR-mix-FL *JCV-BKV*,**
   - **5 µl** of mixture of **PCR-buffer-B** and **polymerase (TaqF)**.

   Prepare the mixture for the total number of test and control samples (see item 8 for the number of control samples) plus extra volume for several reactions.

**NOTE:** Reaction mixture components should be mixed just before analysis.

3. Thaw the content of the tubes with **PCR-mix-FL *JCV-BKV*** and the mixture of **PCR-buffer-B** and **polymerase (TaqF)**. Sediment the drops on vortex.
4. To prepare the reaction mixture, mix the required quantity of **PCR-mix-FL *JCV-BKV*** and and the mixture of **PCR-buffer-B** and **polymerase (TaqF)** in a new sterile tube**.** Sediment the drops on vortex.
5. Take the required number of tubes/strips for amplification of the DNA obtained from test and control samples.
6. Transfer **15 µl** of the prepared mixture to each tube. Utilize the rest of reaction mixture.
7. Add **15 µl** of reaction mixture into each tube.
8. Carry out the control reactions:

**C1** — Add **10 µl** of **C1 *JCV-BKV*** to two tubes with reaction mixture

**C2** — Add **10 µl** of **C2 *JCV-BKV*** to two tubes with reaction mixture

**C-** — Add **10 µl** of **the sample extracted from the Negative Control (C-) reagent** to the tube with reaction mixture

**PCE** — Add **10 µl** of **the sample extracted from Positive Control *JCV-BKV*** to the tube with reaction mixture

**NOTE:** It is also necessary to carry out Negative Control of Amplification (NCA) at suspicion on possible contamination

**NCA** – Add **10 µl** of **TE-buffer** to the tube with reaction mixture

#### 8.2.2. Amplification

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

Table 2

**AmpliSens unified amplification program for rotor-[1] and plate-type[2] instruments**

| Step | Temperature, °C | Time | Fluorescence detection | Cycles |
|---|---|---|---|---|
| 1 | 50 | 15 min | – | 1 |
| 2 | 95 | 15 min | – | 1 |
| 3 | 95 | 10 s | – | 45 |
| | 60 | 20 s | FAM, JOE, ROX | |

**NOTE:** Any combination of the tests including test with reverse transcription and amplification can be performed in one instrument simultaneously with the use of the unified amplification program. If several tests in "multiprime" format are carried out simultaneously, the detection is enabled in other used channels except for the specified ones. If only the tests for pathogen agent DNA detection are performed in one instrument then the first step of reverse transcription (50 °C – 15 minutes) can be omitted for time saving

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

**NOTE:** It is recommended to sediment drops from walls of tubes by short centrifugation 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.

[1] For example, Rotor-Gene Q (QIAGEN, Germany) or equivalent.
[2] For example, CFX96 (Bio-Rad, USA) or equivalent.

## 9. DATA ANALYSIS

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

Table 3

| Channel for the fluorophore | FAM | JOE | ROX |
|---|---|---|---|
| Signal registration, indicating the amplification product accumulation | Internal Control-FL (IC) DNA | *JCV* DNA | *BKV* DNA |

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the cDNA sample in the corresponding column of the results grid.

Based on the obtained *Ct* values and specified concentration values of DNA calibrators (C1 and C2) a calibration line is automatically plotted and the concentration values of *JCV* DNA, *BKV* DNA and Internal Control-FL (IC) DNA in copies/reaction are calculated.

The concentration of *JCV* DNA and *BKV* DNA per 1 ml of test sample is calculated according to the formula:

$$\frac{\textbf{number of } \textit{JCV} \textbf{ (or } \textit{BKV}\textbf{) DNA copies per reaction}}{\textbf{number of Internal Control-FL (IC) DNA copies per reaction}} \times \textbf{coefficient A} \times \textbf{coefficient B} = \textbf{copies/ml}$$

where;

**Coefficient A** is a coefficient that takes into account the volume of test sample before pretreatment. It can be calculated according to the formula:

$$\textbf{Coefficient A} = \frac{\textbf{100}}{\textbf{the volume of test sample before pretreatment (µl)}}$$

**Coefficient B** is number of IC copies in 1 ml of the test sample. The coefficient takes into account the DNA loss during the extraction procedure.

**NOTE:** Concentration values of calibrators and coefficient B are specified in the *Important Product Information Bulletin* enclosed to the PCR kit. They are specific for each lot and cannot be used with PCR kits of other lots.

Table 4

**Results interpretation for the test samples**

| Result | Interpretation |
|---|---|
| Invalid | The *Ct* value in the channel for the FAM fluorophore is absent or determined greater than the boundary value. The PCR analysis (beginning with the DNA extraction stage) should be repeated for this sample |
| *JCV* and/or *BKV* DNA is not detected | The *Ct* value for *JCV* and/or *BKV* DNA is absent and the *Ct* value determined in the channel for the FAM fluorophore is less than the boundary value. The result is ***JCV* and/or *BKV* DNA is not detected** |
| less than $1x10^3$ copies/ml | *JCV* and/or *BKV* DNA was detected in concentration less than the linear measurement range of the PCR kit. The result is **less than $1x10^3$ *JCV* and/or *BKV* DNA copies/ml** |
| X x $10^y$ copies/ml | Calculated concentration value (copies/ml) is in the linear measurement range of the PCR kit. The result is ***JCV* and/or *BKV* DNA is detected in concentration X x $10^y$ copies/ml** |
| greater than $1x10^8$ copies/ml | *JCV* and/or *BKV* DNA was detected in concentration greater than the linear measurement range of the PCR kit. The result is **greater than $1x10^8$ *JCV* and/or *BKV* DNA copies/ml** |

**The results of the analysis is considered reliable only if the results obtained for controls of amplification and extraction stages are correct (according to Table 5 and the *Important Product Information Bulletin* enclosed to the PCR kit).**

Table 5

**Results for controls**

| Control | Stage for control | Amplification results in the channel for fluorophore | | |
|---|---|---|---|---|
| | | FAM | JOE | ROX |
| PCE | DNA extraction | *Ct* value is < boundary value | *Ct* value is < boundary value, concentration value is within the range | *Ct* value is < boundary value, concentration value is within the range |
| C- | DNA extraction | *Ct* value is < boundary value | *Ct* value is absent | *Ct* value is absent |
| NCA | PCR | *Ct* value is absent | *Ct* value is absent | *Ct* value is absent |
| C1 | PCR | *Ct* value is **defined** | *Ct* value is **defined** | *Ct* value is **defined** |
| C2 | PCR | *Ct* value is **defined** | *Ct* value is **defined** | *Ct* value is **defined** |

**NOTE:** Boundary *Ct* values and the range of Positive Control *JCV-BKV* concentration are specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

## 10. TROUBLESHOOTING

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

1. The *Ct* value determined for the Positive Control of Extraction (PCE) in the channels for the FAM and/or JOE and/or ROX fluorophores is greater than the boundary *Ct* value or absent. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples.
2. The calculated concentration of the Positive Control *JCV-BKV* does not fit in the range specified in the *Important Product Information Bulletin*. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples.
3. The *Ct* value is determined for the Negative Control of Extraction (C–) in the channels for the JOE and/or ROX fluorophores. The contamination of laboratory with amplification fragments 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 PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples in which specific DNA was detected.
4. The *Ct* value is determined for the Negative Control of amplification (NCA) in the channels for the FAM and/or JOE and/or ROX fluorophores. The contamination of laboratory with amplification fragments 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 samples in which specific DNA was detected.
5. The *Ct* values are absent for the DNA-calibrators C1 and C2 in either of the specified channels for fluorophores. The amplification and detection should be repeated for all the samples.
6. The correlation coefficient $R^2$ is less than 0.98 when plotting the calibration curve. Check the correctness of set concentrations of calibrators in accordance with the *Important Product Information Bulletin*. If the improper result has been obtained again the amplification and detection for all the samples should be repeated.
7. The *Ct* value is determined for the test sample, whereas the area of typical exponential growth of fluorescence is absent (the graphic looks like approximate straight line). It is necessary to check the correctness of selected threshold line level or parameters of base line calculation. If the result has been obtained with the correct level of threshold line (base line), the amplification and detection should be repeated for this sample.

If you have any further questions or if you encounter problems, please contact our Authorized representative in the European Community.

## 11. TRANSPORTATION

**AmpliSens® *JCV-BKV* screen/monitor-**FRT PCR kit should be transported at 2–8 °C for no longer than 5 days.

## 12. STABILITY AND STORAGE

All components of the **AmpliSens® *JCV-BKV* screen/monitor-FRT** PCR kit are to be stored at 2–8 °C when not in use (except for PCR-buffer-B, polymerase (TaqF) and PCR-mix-FL *JCV-BKV*). All components of the **AmpliSens® *JCV-BKV* screen/monitor-FRT** PCR kit are stable until labeled expiration date. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.

**NOTE:** PCR-buffer-B, polymerase (TaqF) and PCR-mix-FL *JCV-BKV* are to be stored at the temperature from minus 24 to minus 16 °C.

**NOTE:** PCR-mix-FL *JCV-BKV* is to be kept away from light.

## 13. SPECIFICATIONS

### 13.1. Linear measurement range and limit of detection

| Test material | The volume of sample for extraction, µl | Nucleic acid extraction kit | PCR kit | Limit of detection, GE/ml | Linear measurement range, copies/ml |
|---|---|---|---|---|---|
| Whole blood | 100 | RIBO-prep | variant FRT-100 F | $5x10^2$ | $1x10^3$ – $1x10^8$ |
| Cerebrospinal fluid (CSF) | | | | | |
| Urine | | | | | |

The claimed features are achieved while respecting the rules specified in the section *Sampling and Handling*.

### 13.2. Analytical specificity

The analytical specificity of **AmpliSens® *JCV-BKV* screen/monitor-FRT** PCR kit is ensured by 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 PCR kit detects the DNA fragments of claimed microorganisms. The analytical specificity was proved on the following strains of microorganisms *Adenovirus*, *Bocavirus, Parvovirus* B19, *Rubella virus, Enterovirus, Metapneumovirus, Coronavirus, Rhinovirus, Parainfluenza virus*, *HSV1* (*herpes simplex virus* type I), *HSV2* (*herpes simplex virus* type II), *CMV* (cytomegalovirus), *EBV* (Epstein-Barr virus), *VZV* (*Varicella-Zoster virus*), *HHV6* (*herpes virus* type 6), *HHV7* (*herpes virus* type 7), *HHV8* (*herpes virus* type 8), *HBV* (hepatitis B virus), *HCV* (hepatitis C virus), *HIV* (*human immunodeficiency virus*), *Influenza virus* A, *Influenza virus* B, respiratory syncytial virus, *JCV* (*JC*-virus), *BKV* (*BK*-virus), *HPV* 6, 11, 16, 18, 31, 33, 39, 45, 51, 52, 56, 58 (*human papilloma virus* of 6, 11, 16, 18, 31, 33, 39, 45, 51, 52, 56, 58 types), *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Streptococcus agalactiae, Streptococcus pyogenes, Streptococcus pneumoniae, Enterococcus faecium, Enterococcus faecalis, Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Listeria monocytogenes, Neisseria meningitidis, Haemophylus influenza, Chlamydia (Chlamydoplila) pneumonia, Mycoplasma pneumonia, Moraxella catarrhalis, Stenotrophomonas maltophilia, Mycobacterium tuberculosis complex, Proteus mirabilis, Toxoplasma gondii, Candida albicans, Candida glabrata, Candida krusei, Cryptococcus neoformans, Pneumocystis jirovecii,* and also human genomic DNA.

The nonspecific responses were not observed while testing the DNA samples of the above mentioned microorganisms, as well as human DNA. The specific of testing was confirmed by sequencing of detected amplified fragments.

The clinical specificity of **AmpliSens® *JCV-BKV* screen/monitor-FRT** PCR kit was confirmed in laboratory clinical trials.

### 13.3. Reproducibility, repeatability and trueness

Repeatability and reproducibility were determined by testing of quality control samples with concentrations $1x10^7$; $1x10^6$ and $1x10^5$ copies/ml.

Table 6

**Reproducibility**

| Micro-organism | Initial concentration value, copies/ml | Number of repeats | Average concentration value, lg | Standard deviation (SD) | Coefficient of variation (CV), % |
|---|---|---|---|---|---|
| *JCV* | $10^7$ | 80 | 7.02 | 0.09 | 1.25 |
| | $10^6$ | 80 | 6.12 | 0.08 | 1.23 |
| | $10^5$ | 80 | 5.15 | 0.08 | 1.56 |
| *BKV* | $10^7$ | 80 | 7.16 | 0.08 | 1.10 |
| | $10^6$ | 80 | 6.11 | 0.07 | 1.13 |
| | $10^5$ | 80 | 5.19 | 0.09 | 1.80 |

Table 7

**Repeatability**

| Micro-organism | Initial concentration value, copies/ml | Number of repeats | Average concentration value, lg | Standard deviation (SD) | Coefficient of variation (CV), % |
|---|---|---|---|---|---|
| ***JCV*** | $10^7$ | 40 | 7.00 | 0.06 | 0.80 |
| | $10^6$ | 40 | 6.09 | 0.05 | 0.76 |
| | $10^5$ | 40 | 5.12 | 0.06 | 1.25 |
| ***BKV*** | $10^7$ | 40 | 7.11 | 0.05 | 0.67 |
| | $10^6$ | 40 | 6.14 | 0.05 | 0.78 |
| | $10^5$ | 40 | 5.16 | 0.08 | 1.53 |

Table 8

**Trueness**

| Micro-organism | Number of repeats | Average value of measurement, lg | Specified value, lg | Bias (B), % |
|---|---|---|---|---|
| *JCV* | 100 | 3.94 | 3.95 | 0.25 |
| *BKV* | 100 | 3.98 | 3.95 | 0.76 |

### 13.4. Diagnostic characteristics

Table 9

**The results of testing AmpliSens® *JCV-BKV* screen/monitor-FRT PCR kit in comparison with the reference assay**

| Detected pathogen | Sample type | The results of application of AmpliSens® *JCV-BKV* screen/monitor-FRT PCR kit | | Results of using the reference assay [3] | |
|---|---|---|---|---|---|
| | | | | Positive | Negative |
| *JCV* | Whole blood | 200 samples were tested | **Positive** | **99** | **0** |
| | | | **Negative** | **1** | **100** |
| | Cerebro-spinal fluid (CSF) | 200 samples were tested | **Positive** | **100** | **0** |
| | | | **Negative** | **0** | **100** |
| *BKV* | Whole blood | 200 samples were tested | **Positive** | **100** | **0** |
| | | | **Negative** | **0** | **100** |
| | Urine | 200 samples were tested | **Positive** | **100** | **0** |
| | | | **Negative** | **0** | **100** |

Diagnostic sensitivity was determined by testing of 100 type samples of each type of biological material (whole blood, cerebrospinal fluid (CSF), urine), which contain dilutions of *JCV* and *BKV* Quality Control Samples. The Sanger sequencing method was used to prove the presence of *JCV* and *BKV* DNA in type samples. *JCV* and *BKV* DNA was detected in initial dilutions of *JCV* and *BKV* Quality Control Samples using sequencing method. Diagnostic specificity was proved by testing of 100 type samples of each type of biological material. The blood and urine samples were taken from conventionally healthy donors, the samples of cerebrospinal fluid (CSF) were collected from the patients with symptoms of purulent meningitis.

Table 10

**Diagnostic characteristics of AmpliSens® *JCV-BKV* screen/monitor-FRT PCR kit**

| Detected pathogen | Sample type | Diagnostic sensitivity[4], in the interval (%) | Diagnostic specificity[5], in the interval (%) |
|---|---|---|---|
| *JCV* | Whole blood | 100 | 100 |
| | Cerebrospinal fluid (CSF) | 100 | 100 |
| *BKV* | Whole blood | 100 | 100 |
| | Urine | 100 | 100 |

## 14. REFERENCES


1. Guidelines to **AmpliSens® *JCV-BKV* screen/monitor-FRT** PCR kit using the PCR instruments with real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science "Central Research Institute for Epidemiology"


## 15. QUALITY CONTROL

In accordance with Federal Budget Institute of Science "Central Research Institute for Epidemiology" ISO 13485-Certified Total Quality Management System, each lot of **AmpliSens® *JCV-BKV* screen/monitor-FRT** PCR kit is tested against predetermined specifications to ensure consistent product quality.

**List of Changes Made in the Instruction Manual**

| VER | Location of changes | Essence of changes |
|---|---|---|
| 25.05.20 KK | Through the text | The text formatting was changed |
| | Footer | The phrase "Not for use in the Russian Federation" was added |
| 11.03.21 MA | — | The name, address and contact information for Authorized representative in the European Community was changed |
| 31.01.22 MM | Through the text | The reference numbers of nucleic acid extraction kits were deleted |

**AmpliSens®**


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[3] The Sanger sequencing method was used as the reference assay.
[4] Relative sensitivity in comparison with applied reference methods.
[5] Relative specificity in comparison with applied reference methods.