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For Professional Use Only

AmpliSens® *Neisseria gonorrhoeae*-screen-FRT PCR kit

Instruction Manual

AmpliSens®

EC REP

Ecoli s.r.o., Studenohorska 12
841 03 Bratislava 47
Slovak Republic
Tel.: +421 2 6478 9336
Fax: +421 2 6478 9040

Federal Budget Institute of Science “Central Research Institute for Epidemiology”
3A Novogireevskaya Street
Moscow 111123 Russia

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1. INTENDED USE

AmpliSens® ***Neisseria gonorrhoeae*-screen-FRT** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Neisseria gonorrhoeae* DNA in clinical materials (urogenital, rectal, and oropharyngeal swabs; conjunctival discharge; prostate gland secretion; and urine samples) by using real-time hybridization-fluorescence detection.

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

2. PRINCIPLE OF PCR DETECTION

Neisseria gonorrhoeae DNA detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *Neisseria gonorrhoeae* primers. In real-time PCR, the amplified product is detected using 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®** ***Neisseria gonorrhoeae*-screen-FRT** PCR kit is a qualitative test that contains the Internal Control (IC). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition. **AmpliSens®** ***Neisseria gonorrhoeae*-screen-FRT** PCR kit uses "hot-start," which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by separation of nucleotides and Taq-polymerase by using a wax layer. Wax melts and reaction components mix only at 95 °C.

3. CONTENT

AmpliSens® ***Neisseria gonorrhoeae*-screen-FRT** PCR kit is produced in 3 forms:

AmpliSens® *Neisseria gonorrhoeae*-screen-FRT PCR kit variant FRT (for use with RG)

REF R-B51(RG)-CE.

AmpliSens® *Neisseria gonorrhoeae*-screen-FRT PCR kit variant FRT (for use with iQ)

REF R-B51(iQ)-CE.

AmpliSens® *Neisseria gonorrhoeae*-screen-FRT PCR kit variant FRT-100 F (for use with RG, iQ) **REF** R-B51-F(RG,iQ)-CE.

AmpliSens® *Neisseria gonorrhoeae*-screen-FRT PCR kit variant FRT includes:

Reagent	***Description***	***Volume (ml)***	***Quantity***
PCR-mix-1-FL *Neisseria gonorrhoeae*-screen (ready-to-use single-dose test tubes (*under wax)*)	colorless clear liquid	0.01	110 tubes of 0.2 ml
PCR-mix-2-FL-red	red clear liquid	1.1	1 tube
Positive Control complex (C+)	colorless clear liquid	0.2	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C-)*	colorless clear liquid	1.2	1 tube
Internal Control-FL (IC)**	colorless clear liquid	1.0	1 tube

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

** add 10 µl of Internal Control-FL during the DNA extraction procedure directly to the sample/lysis mixture (see DNA-sorb-AM **REF** K1-12-100-CE protocol).

AmpliSens® *Neisseria gonorrhoeae*-screen-FRT PCR kit is intended for 110 reactions (including controls).

AmpliSens® *Neisseria gonorrhoeae*-screen-FRT PCR kit variant FRT-100 F includes:

Reagent	***Description***	***Volume (ml)***	***Quantity***
PCR-mix-1-FL *Neisseria gonorrhoeae*-screen	colorless clear liquid	1.2	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	2 tube
Positive Control complex (C+)	colorless clear liquid	0.2	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C-)*	colorless clear liquid	1.2	1 tube
Internal Control-FL (IC)**	colorless clear liquid	1.0	1 tube

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

** add 10 µl of Internal Control-FL during the DNA extraction procedure directly to the sample/lysis mixture (see DNA-sorb-AM **REF** K1-12-100-CE protocol).

AmpliSens® *Neisseria gonorrhoeae*-screen-FRT PCR kit is intended for 110 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit
- Transport medium.

- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml tubes.
- PCR box.
- Personal thermocyclers (for example, Rotor-Gene™ 3000 or Rotor-Gene™ 6000 (Corbett Research, Australia); Rotor-Gene Q (Qiagen, Germany); iCycler iQ or iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA); or equivalent).
- Disposable polypropylene microtubes for PCR (0.1- or 0.2-ml; for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °C.
- Waste bin for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, 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 a kit after its expiration date.
- Dispose of all samples and unused reagents in compliance with local authorities' requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant, such as 0.5% sodium hypochlorite or another suitable disinfectant.
- Avoid contact with the skin, eyes and mucosa. If skin, eyes and mucosa contact immediately flush with water, seek medical attention.
- Material Safety Data Sheets (MSDS) are available on request.

- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which 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

Obtaining samples of biological materials for PCR-analysis, transportation and storage are described in the manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® *Neisseria gonorrhoeae*-screen-FRT PCR kit is intended for the analysis of DNA extracted by DNA extraction kits from urogenital swabs, urine sediment (use the first portion of the morning specimen), or the prostate gland secretion.

7. WORKING CONDITIONS

AmpliSens® *Neisseria gonorrhoeae*-screen-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA extraction

It is recommended to use the following nucleic acid extraction kits:

- DNA-sorb-AM, **REF** K1-12-100-CE.

Carry out the DNA extraction according to the manufacturer's instructions.

8.2. Preparing PCR

8.2.1. Preparing tubes for PCR

Variant FRT

The total reaction volume is **30 µl**, the volume of DNA sample is **10 µl**.

1. Prepare the required number of tubes with **PCR-mix-1-FL *Neisseria gonorrhoeae*-screen** for amplification of DNA from clinical and control samples.
2. Add **10 µl** of **PCR-mix-2-FL-red** to the surface of the wax layer of each tube ensuring that it does not fall under the wax and mix with **PCR-mix-1-FL *Neisseria gonorrhoeae*-screen**.
3. Add **10 µl** of **DNA** obtained from clinical or control samples at the DNA extraction stage into the prepared tubes using tips with aerosol barrier.

4. Carry out the control amplification reactions:

NCA - Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).

C+ - Add **10 µl** of **Positive Control complex** to the tube labeled C+ (Positive Control of Amplification).

C– - Add **10 µl** of sample isolated from **Negative Control** to the tube labeled C- (Negative Control of Extraction).

Variant FRT-100 F

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

1. Thaw the tube with **PCR-mix-2-FRT**. Prepare the required number of tubes with **PCR-mix-1-FL *Neisseria gonorrhoeae*-screen**, **PCR-mix-2-FRT,** and **Polymerase (TaqF)** and sediment drops by short centrifugation (1-2 s).
2. Prepare the required quantity of tubes or strips for amplification of DNA from clinical and control samples.
3. For carrying out N reactions (including 2 controls), mix in a new tube **10*(N+1) µl of PCR-mix-1-FL *Neisseria gonorrhoeae*-screen, 5.0*(N+1) µl of PCR-mix-2-FRT,** and **0.5*(N+1) µl of polymerase (TaqF)**.
4. Mix and precipitate the drops by short centrifuging.
5. Transfer **15 µl** of the prepared mixture into each tube.
6. Add **10 µl** of **DNA** obtained from clinical or control samples at the DNA extraction stage into the prepared tubes using tips with aerosol barrier.
7. Carry out the control amplification reactions:

NCA - Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).

C+ - Add **10 µl** of **Positive Control complex** to the tube labeled C+ (Positive Control of Amplification).

C– - Add **10 µl** of sample isolated from **Negative Control** to the tube labeled C- (Negative Control of Extraction).

8.2.2 Amplification

Program the real-time amplification instrument according to manufacturer's manual and Guidelines [2].

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

AmpliSens-1 program

	Rotor-type Instruments[1]			Plate-type Instruments[2]		
Step	***Temperature, °C***	***Time***	**Cycles**	***Temperature, °C***	***Time***	**Cycles**
1	**95**	15 min	1	**95**	15 min	1
2	**95**	5 s	5	**95**	5 s	5
	60	20 s		**60**	20 s	
	72	15 s		**72**	15 s	
3	**95**	5 s	40	**95**	5 s	40
	60	20 s *fluorescent signal detection*		**60**	30 s *fluorescent signal detection*	
	72	15 s		**72**	15 s	

Fluorescent signal is detected in the channels designed for the FAM/Green and JOE/Yellow/HEX fluorophores (other channels are enabled if several tests are simultaneously carried out in a single run).

2. Adjust the fluorescence channel sensitivity according to *Important Product Information Bulletin.*
3. Insert tubes into the reaction module of the device.
4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

The results are interpreted by the software of the used Instrument by the crossing (or not crossing) of the fluorescence curve with the threshold line.

The Internal Control DNA is detected in the JOE fluorescence channel, the *Neisseria gonorrhoeae* DNA is detected in the FAM fluorescence channel.

See the **Manufacturer's manual, Guidelines** and **Important product information bulletin** for data analysis settings.

Principle of interpretation:

The results are interpreted by the crossing (or not crossing) of the fluorescence curve with the threshold line.

- *Neisseria gonorrhoeae* DNA is **detected** in a sample if its Ct value is detected in the results grid in the FAM channel.
- *Neisseria gonorrhoeae* DNA is **not detected** if its Ct value is not detected in the results grid (the fluorescence curve does not cross the threshold line) in the FAM channel and the Ct value in the results grid in the JOE channel does not exceed the threshold Ct

[1] For example,Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q or equivalent.
[2] For example, iCycler, iQ5, Mx3000P, Mx3000 or equivalent.

value.

- The analysis result is **invalid** if the Ct value is not detected in the results grid (the fluorescence curve does not cross the threshold line) in FAM channel and the Ct value in the results grid in the JOE channel is not detected or exceeds the threshold Ct value. In such cases, PCR should be repeated.

The result of the analysis is considered to be reliable only if the results obtained for both Positive and Negative Controls of amplification as well as Negative Control of extraction are correct (see Table 2).

Table 2

Results for controls

Control	Stage for control	Ct value in channel		Interpretation
		FAM	JOE	
C–	DNA isolation	Neg	Pos (<boundary value)*	OK
NCA	Amplification	Neg	Neg	OK
C+	Amplification	Pos (<boundary value)	Pos (<boundary value)	OK

*For boundary Ct values of the samples, Negative Control of Extraction and Positive Control of Amplification, see the **Important Product Information Bulletin**.

10. TROUBLESHOOTING

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

1. If, for the positive control of amplification (C+), the Ct value in the FAM channel is absent of exceeds the boundary Ct value, repeat amplification of all samples in which *Neisseria gonorrhoeae* DNA was not detected.
2. If, for the negative control of DNA extraction (C–) and/or negative control of amplification (NCA), a Ct value in the FAM channel was detected, repeat PCR analysis of all samples in which *Neisseria gonorrhoeae* DNA was detected starting from the DNA extraction stage.
3. If the Ct value is absent in JOE/Yellow/HEX and FAM/Green channels or the Ct value in the JOE/Yellow /HEX channel is greater than the specified boundary Ct value, PCR should be repeated. If the same result is obtained, the extraction stage for the sample should be repeated. If the IC signal of this sample was detected normally in any other PCR test, it is not necessary to repeat the extraction stage (for iCycler iQ or iQ5 instruments).
4. If a Ct value is present for C– in the FAM/Green channel and/or for NCA in the FAM, JOE/Yellow /HEX channels in the results grid, this indicates contamination of reagents or samples. In such cases, the results of analysis must be considered as invalid. Test

analysis must be repeated and measures to detect and eliminate the source of contamination must be taken.

5. If no signal is detected for the Positive Controls of amplification, this may suggest that the programming of the temperature profile of the used Instrument was incorrect, or that the configuration of the PCR reaction was incorrect, or that the storage conditions for kit components did not comply with the manufacturer's instruction, or that the reagent kit has expired. Programming of the used instrument, storage conditions, and the expiration date of reagents should be checked, and then PCR should be repeated.
6. If a positive result (the fluorescence curve crosses the threshold line) is detected for a sample with a fluorescence curve without the typical exponential growth phase (the curve is linear), this may suggest incorrect setting of the threshold line or incorrect calculation of baseline parameters. Such a result should not be considered as positive. Once the threshold line has been set correctly, PCR analysis of the sample should be repeated (if iCycler iQ or iQ5 instruments are used).

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

11. TRANSPORTATION

AmpliSens® *Neisseria gonorrhoeae*-screen-FRT PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *Neisseria gonorrhoeae*-screen-FRT** PCR kit (except for polymerase (TaqF) and PCR-mix-2-FRT) are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® *Neisseria gonorrhoeae*-screen-FRT** PCR kit are stable until the expiration date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

Polymerase (TaqF) and PCR-mix-2-FRT are to be stored at temperature from minus 24 to minus 16 °C when not in use.

PCR-mix-1-FL *Neisseria gonorrhoeae*-screen should be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Analytical Sensitivity of **AmpliSens® *Neisseria gonorrhoeae*-screen-FRT** PCR kit is specified in the table below.

Clinical material	Transport medium	Nucleic acid extraction kit	Sensitivity, GE/ml [3]
Urogenital swabs	Transport Medium for Swabs (**REF** 956-CE, **REF** 987-CE) or Transport Medium with Mucolytic (**REF** 952-CE, **REF** 953-CE)	DNA-sorb-AM	5×10^2
Urine (pretreatment is required)	—	DNA-sorb-AM	1×10^3

13.2. Specificity

The analytical specificity of **AmpliSens® *Neisseria gonorrhoeae*-screen-FRT** PCR kit is ensured by selection of specific primers and probes as well as the selection of strict reaction conditions. The primers and probes were checked for possible homologies to all sequences deposited in gene banks by sequence comparison analysis. Nonspecific reactions were absent in tests with human DNA samples and a DNA panel of following microorganisms: *Neisseria flava, N.subflava, N.sicca, N.mucosa, N.lactamica,* and *N.meningitides; Gardnerella vaginalis; Lactobacillus* spp.; *Escherichia coli; Staphylococcus aureus; Streptococcus pyogenes and S. agalactia; Candida albicans; Mycoplasma hominis* and *M.genitalium; Ureaplasma urealyticum* and *U.parvum; Chlamydia trachomatis; Treponema pallidum; Trichomonas vaginalis; Toxoplasma gondii; HSV* types 1 and 2; *CMV;* and *HPV*. The clinical specificity of **AmpliSens® *Neisseria gonorrhoeae*-screen-FRT** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2008.
2. Guidelines “Real-Time PCR Detection of STIs and Other Reproductive Tract Infections”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow.

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 **AmpliSens®**

[3] Genome equivalents (GE) of the microorganism per 1 ml of the sample placed in the transport medium specified.

***Neisseria gonorrhoeae*-screen-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
IVD	*In vitro* diagnostic medical device		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation		Keep away from sunlight
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C–**	Negative control of extraction
EC REP	Authorised representative in the European Community	**C+**	Positive control of amplification
FBIS CRIE	Federal Budget Institute of Science “Central Research Institute for Epidemiology”	**IC**	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
02.07.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”