



For *in Vitro* Diagnostic Use

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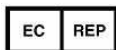
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AmpliSens[®] *Neisseria gonorrhoeae*-test-FRT

PCR kit

Instruction Manual

AmpliSens[®]



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1. INTENDED USE.

AmpliSens® Neisseria gonorrhoeae-test-FRT PCR kit is an in vitro nucleic acid amplification test for qualitative detection of *Neisseria gonorrhoeae* DNA in the human clinical materials (cervical, urethral scrapes (swabs), scrapes from oropharynx, scrapes from anorectal region, urine sediment, secrete of the prostate gland, conjunctival swabs, synovial fluid) by using real-time hybridization-fluorescence detection.

2. PRINCIPLE OF PCR DETECTION.

Neisseria gonorrhoeae detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *Neisseria gonorrhoeae* primers. In real-time PCR the amplified product is detected using fluorescent dyes. These dyes are usually linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time PCR monitoring of the 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-test-FRT** PCR kit is a qualitative test, which contain the Internal Control (IC). It must be used in the isolation procedure in order to control the isolation process of each individual sample and to identify possible reaction inhibition. **AmpliSens® Neisseria gonorrhoeae-test-FRT** PCR kit uses “hot-start”, which greatly reduces frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using wax layer (for FRT-variant). Wax melting and reaction mix components occur only at 95°C. In variant FRT-100F “hot-start” is guaranteed by application of polymerase (TaqF). Chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT.

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

AmpliSens® *Neisseria gonorrhoeae*-test-FRT PCR kit variant FRT for use with RG **REF** R-B56(RG)-CE.

AmpliSens® *Neisseria gonorrhoeae*-test-FRT PCR kit variant FRT for use with iQ **REF** R-B56(iQ)-CE.

AmpliSens® *Neisseria gonorrhoeae*-test-FRT PCR kit variant FRT-100F **REF** R-B56-F(RG, iQ)-CE.

AmpliSens® Neisseria gonorrhoeae-test-FRT PCR kit, variant FRT includes:

| Reagent | Description | Volume (ml) | Quantity |
|--|------------------------|-------------|---------------------|
| PCR-mix-1-FEP/FRT <i>Neisseria gonorrhoeae</i>-test (under wax) | colorless, clear fluid | 0.008 | 110 tubes of 0.2 ml |
| PCR-mix-2-FL | colorless, clear fluid | 0.77 | 1 tube |
| Positive Control DNA <i>Neisseria gonorrhoeae</i> (C+) | colorless, clear fluid | 0.2 | 1 tube |
| DNA-buffer | colorless, clear fluid | 0.5 | 1 tube |
| Negative Control (C-)* | colorless, clear fluid | 1.2 | 1 tube |
| Internal Control-FL (IC)** | colorless, clear fluid | 1.0 | 1 tube |

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

** add 10 µl of Internal Control-FL during the DNA isolation procedure directly to the sample/lysis mixture (see DNA-sorb-AM **REF** K1-12-100-CE or DNA-sorb-B **REF** K1-2-100-CE)

AmpliSens® Neisseria gonorrhoeae-test-FRT PCR kit, variant FRT is sufficient for 110 reactions, including controls.

AmpliSens® Neisseria gonorrhoeae-test-FRT PCR kit, variant FRT-100 F includes:

| Reagent | Description | Volume (ml) | Quantity |
|--|------------------------|-------------|----------|
| PCR-mix-1-FEP/FRT <i>Neisseria gonorrhoeae</i>-test (under wax) | colorless, clear fluid | 1.2 | 1 tube |
| PCR-mix-2-FRT | colorless, clear fluid | 0.3 | 2 tubes |
| Positive Control DNA <i>Neisseria gonorrhoeae</i> (C+) | colorless, clear fluid | 0.2 | 1 tube |
| Polymerase (TaqF) | colorless, clear fluid | 0.06 | 1 tube |
| DNA-buffer | colorless, clear fluid | 0.5 | 1 tube |
| Negative Control (C-)* | colorless, clear fluid | 1.2 | 1 tube |
| Internal Control-FL (IC)** | colorless, clear fluid | 1.0 | 1 tube |

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

** add 10 µl of Internal Control-FL during the DNA isolation procedure directly to the sample/lysis mixture (see DNA-sorb-AM **REF** K1-12-100-CE or DNA-sorb-B **REF** K1-2-100-CE)

AmpliSens® Neisseria gonorrhoeae-test-FRT PCR kit, variant FRT-100 F is sufficient for 110 reactions, including controls.

4. ADDITIONAL REQUIREMENTS.

- DNA isolation kit
- 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 (frequency no more than 16000 rpm) with rotor for 2 ml reaction tubes
- PCR box
- Personal thermocyclers (for example, «Rotor-Gene» 3000/6000 («Corbett Research», Australia), «iQ iCycler», «iQ5» («BioRad», USA) or equivalent)
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity (for example, “Axygen”, USA)
- Refrigerator for temperature between 2 and 8 °C
- Deep-freezer with temperature no more than minus 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 specimens, controls and 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 other 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, move 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.



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

AmpliSens® *Neisseria gonorrhoeae*-test-FRT PCR kit is intended for the analysis of DNA extracted by DNA isolation kits from cervical, urethral scrapes (swabs), scrapes from oropharynx, scrapes from anorectal region, urine sediment (use the first portion of the morning specimen), secrete of the prostate gland, conjunctival swabs, synovial fluid.

7. PROTOCOL.

7.1. DNA Isolation

It’s recommended to use the following nucleic acid extraction kits:

- “DNA-sorb-AM”, **REF** K1-12-100-CE (for cervical, urethral scrapes (swabs), scrapes from oropharynx, scrapes from anorectal region, urine sediment, conjunctival swabs, synovial fluid)
- “DNA-sorb-B”, **REF** K1-2-100-CE (for secrete of the prostate gland).



Carry the DNA isolation according to the manufacturer’s instructions.

7.2. Preparing the PCR.

Total reaction volume - **25 µl**, volume of DNA sample - **10 µl**.

7.2.1 Preparing tubes for PCR.

Variant FRT

1. Prepare the required number of the tubes with **PCR-mix-1-FEP/FRT *Neisseria gonorrhoeae*-test** and wax for amplification of DNA from clinical and control samples.
2. Add **7 µl** of **PCR-mix-2-FL** 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-FEP/FRT *Neisseria gonorrhoeae*-test**.

Variant FRT-100 F

1. Prepare the required number of the tubes for amplification of DNA from clinical and control samples (0.2 ml tubes for 36-Well rotor or 0.1 ml stripes for 72-Well rotor).
2. For carrying of N reactions (including 2 controls) mix in a new tube **10*(N+1) µl** of **PCR-mix-1-**

FEP/FRT *Neisseria gonorrhoeae*-test, 5.0*(N+1) µl of PCR-mix-2-FRT and 0.5*(N+1) µl of polymerase (TaqF). Vortex the tube, then centrifuge shortly. Transfer **15 µl** of prepared mix into each tube.

Steps 3 and 4 are effective for both variants.

- Using tips with aerosol barrier add **10 µl** of **DNA samples** isolated from clinical or control samples at the DNA extraction stage into prepared tubes.



The tubes with PCR-mix-1-FEP/FRT *Neisseria gonorrhoeae*-test that are not used at the moment should be stored away from light.

- Carry 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 DNA *Neisseria gonorrhoeae*** to the tube labeled C+ (Positive Control of Amplification).

7.2.2. Amplification.

7.2.2.1. RG

- Program the Rotor-Gene™ according to manufacturer's manual and Appendix 1.
- Create a temperature profile on your Rotor-Gene™ instrument as follows:

AmpliSens-1 RG program

| Step | Temperature, °C | Time | Fluorescence detection | Cycle repeats |
|-----------|-----------------|--------|------------------------|---------------|
| Hold | 95 | 15 min | – | 1 |
| Cycling | 95 | 5 sec | – | 5 |
| | 60 | 20 sec | – | |
| | 72 | 15 sec | – | |
| Cycling 2 | 95 | 5 sec | – | 40 |
| | 60 | 20 sec | FAM/Green, JOE/Yellow | |
| | 72 | 15 sec | – | |



AmpliSens-1 RG general program allows simultaneous conducting of any combination of tests for detection of sexually transmitted diseases pathogens DNA including tests for identifying of *Human Papillomaviruses* (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology) by means of AmpliSens HPV HCR PCR kits.

- Fluorescence detection on fluorometer channels, FAM/Green and JOE/Yellow, is on the 2-nd pass (**60°C**).

- Make the adjustment of the fluorescence channel sensitivity according to Appendix 1.

7.2.2.2. iQ

- Program the iQ™ according to manufacturer's manual and Appendix 2.
- Create a temperature profile on your iQ™ instrument as follows:

AmpliSens-1 iQ program

| Step | Temperature, °C | Time | Fluorescence detection | Cycle repeats |
|-----------|-----------------|--------|------------------------|---------------|
| Hold | 95 | 15 min | – | 1 |
| Cycling | 95 | 5 sec | – | 5 |
| | 60 | 20 sec | – | |
| | 72 | 15 sec | – | |
| Cycling 2 | 95 | 20 sec | – | 40 |
| | 60 | 30 sec | FAM-490, HEX-530 | |
| | 72 | 25 sec | – | |



AmpliSens-1 iQ general program allows a combination of test for the detection of pathogens of sexually transmitted diseases to be carried out simultaneously including tests for the identification of *Human Papillomaviruses* by means of AmpliSens HPV HCR PCR kits.

- Fluorescence detection on fluorometer channels, FAM/Green and JOE/Yellow, is on the 2-nd pass (**60°C**).
- Make the adjustment of the fluorescence channel sensitivity according to Appendix 2.

8. DATA ANALYSIS.

RG. Internal Control is detected on the JOE/Yellow fluorescence channel, *Neisseria gonorrhoeae* DNA is detected on the FAM/Green fluorescence channel.

See **Appendix 1** for data analysis settings for Rotor-Gene™ 3000 or Rotor-Gene™ 6000.

iQ. Internal Control is detected on the HEX fluorescence channel, *Neisseria gonorrhoeae* DNA is detected on the FAM fluorescence channel.

See **Appendix 2** for data analysis settings for iQ5 or iQ iCycler.

8.1. Results interpretation.

8.1.1. RG

The results are interpreted with the software of Rotor-Gene™ 3000 or Rotor-Gene™ 6000 Instrument through the presence (or absence) of crossing of fluorescence curve with the threshold line.

Results for controls

| Control | Stage for control | Ct in channel | | Interpretation |
|---------|-------------------|---------------|------------|----------------|
| | | FAM /Green | JOE/Yellow | |
| C- | DNA isolation | Neg | Pos (X*) | OK |
| NCA | Amplification | Neg | Neg | OK |
| C+ | Amplification | Pos (Z*) | Neg | OK |

*For X, Z values see Appendix 1.

1. The sample is considered as positive for *Neisseria gonorrhoeae* if its Ct value is defined in the results grid on FAM/Green channel. Moreover, the fluorescence curve of the sample should cross the threshold line at the region of typical exponential growth of fluorescence.

2. The sample is considered as negative for *Neisseria gonorrhoeae* if its Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) on FAM/Green channel and in the results grid on the JOE/Yellow channel the Ct value doesn't exceed X.

Results are accepted as relevant if both positive and negative controls of amplification along with negative control of extraction are passed.

8.1.2. iQ

The results are interpreted with the software of iQ5 or iQ iCycler instrument through the presence (or absence) of crossing of fluorescence curve with the threshold line.

Results for controls

| Control | Stage for control | Ct in channel | | Interpretation |
|---------|-------------------|---------------|----------|----------------|
| | | FAM | HEX | |
| C- | DNA isolation | Neg | Pos (X*) | OK |
| NCA | Amplification | Neg | Neg | OK |
| C+ | Amplification | Pos (Z*) | Neg | OK |

*For X, Z values see Appendix 2.

1. The sample is considered as positive for *Neisseria gonorrhoeae* if its Ct value is defined in the results grid on FAM channel. Moreover, the fluorescence curve of the sample should cross the threshold line at the region of typical exponential growth of fluorescence.

2. The sample is considered as negative for *Neisseria gonorrhoeae* if **N/A** appears in results grid on FAM channel (crossing of the fluorescence curve and the threshold line has not been detected) and in the results grid on HEX channel the Ct value does not exceed X.

Results are accepted as relevant if both positive and negative controls of amplification along with negative control of extraction are passed.

9. TROUBLESHOOTING.

Results of analysis are not being registered in the following cases:

1. If no signal is detected for Positive Controls of amplification, it can suggest of incorrect programming of the temperature profile of the instrument, incorrect configuration of the PCR or storage conditions for kit components has not complied with manufacturer instruction, or the reagents kit has expired. It is necessary to ensure adequate programming of the instrument (see 7.2.2.1), storage conditions, and check the expiration date of the reagents, and then repeat PCR once again.
2. If positive signal is registered in Negative Controls (C-, NCA) it indicates the contamination of reagents or samples. In this case results of the analysis for all samples are considered as invalid. Test analysis must be repeated and measures to detect and eliminate the source of contamination are to be taken.
3. If Ct value is absent on both channels, JOE/Yellow and FAM/Green, or the Ct value in JOE/Yellow channel is higher than X, it is necessary to repeat PCR once again.
4. If positive result (fluorescence curve crosses the threshold line) is registered for the sample that has fluorescence curve without typical exponential growth (graph is linear). It can suggest about incorrect threshold line setting or incorrect calculation of base line parameters. Such a result should not be considered as positive. If threshold line was set correctly, the PCR should be repeated for the sample.

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

10. STABILITY AND STORAGE.

All components of the **AmpliSens® *Neisseria gonorrhoeae*-test-FRT** PCR kit (except for Polymerase (TaqF) and PCR-mix-2-FRT) are to be stored at the temperature between 2 °C and 8 °C, when not in use. All components of the **AmpliSens® *Neisseria gonorrhoeae*-test-FRT** PCR kit are to be stable until labeled expiration date.



Polymerase (TaqF) and PCR-mix-2-FRT are to be stored at the temperature no more than minus 16°C.



PCR-mix-1-FEP/FRT *Neisseria gonorrhoeae*-test is to be stored in the place protected from light.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens[®] Neisseria gonorrhoeae-test-FRT** PCR kit is no less than 1×10^3 genome equivalents per 1 ml of sample (GE/ml).



The claimed analytical features of **AmpliSens[®] Neisseria gonorrhoeae-test-FRT** PCR kit are guaranteed only when additional reagents kits “DNA-sorb-AM” or “DNA-sorb-B” (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology) are used.

11.2. Specificity.

Specificity of **AmpliSens[®] Neisseria gonorrhoeae-test-FRT** PCR kit is assured 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 in gene banks published sequences by sequence comparison analysis. Specificity of **AmpliSens[®] Neisseria gonorrhoeae-test-FRT** PCR kit was confirmed in laboratory clinical trials.
















12. REFERENCES.

Handbook “Sampling, transportation, storage of clinical material for PCR diagnostics”, developed by Federal State Institution of Science Central Research Institute of Epidemiology of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2008.

13. QUALITY CONTROL.

In compliance with Federal State Institution of Science “Central Research Institute of Epidemiology” ISO 13485 – certified Quality Management System, each lot of **AmpliSens[®] Neisseria gonorrhoeae-test-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

14. EXPLANATION OF SYMBOLS.

| | | | |
|---|------------------------------------|---|---|
|  | Manufacturer |  | Temperature limitation |
|  | Use by |  | Batch code |
|  | For <i>in Vitro</i> Diagnostic Use |  | Version |
|  | Catalogue number |  | Negative Control of Amplification |
|  | Contains sufficient for <n> tests |  | Authorised representative in the European Community |
|  | Consult instructions for use |  | Caution, consult accompanying documents |
|  | Positive Control of Amplification |  | Negative control of Extraction |
|  | Internal Control | | |