GeneProof
Molecular diagnostics for your routine

GeneProof CT/NG/MG Multiplex PCR Kit

CE 1023 IVD

In vitro diagnostic medical device

The kit has been manufactured according to EC Directive 98/79/EC as an *in vitro* diagnostic medical device and it has been designed for professional use in specialized clinical and research laboratories.

KIT CONTENT

	ISIN Version IS included in the MasterMix			ISEX Version IS supplied in a separate tube Nucleic acid extraction and PCR inhibition control		
REF	**CNMX/ISIN/025** 25 rxn	**CNMX/ISIN/050** 50 rxn	**CNMX/ISIN/100** 100 rxn	**CNMX/ISEX/025** 25 rxn	**CNMX/ISEX/050** 50 rxn	**CNMX/ISEX/100** 100 rxn
MasterMix CNMX	1x750 µl	2x750 µl	4x750 µl	1x750 µl	2x750 µl	4x750 µl
Positive Control CNMX	1x200 µl	1x200 µl	2x200 µl	1x200 µl	1x200 µl	2x200 µl
Internal Standard CNMX	-	-	-	1x1000 µl	1x1000 µl	2x1000 µl

STORAGE AND TRANSPORTATION CONDITIONS

The kit could be transported at temperature below -20 °C. The kit will remain stable at least until the expiry date printed on the package, if the storage temperature is kept (-20 ± 5 °C). The components are stable for a maximum of 3 repeated freezing / thawing cycles after the first use of a particular vial. The component must be used before the expiry date or 14 days after the first use of a particular vial (whichever comes first).

TECHNICAL SPECIFICATION

Target Sequence	the cryptic plasmid sequence and the 16S rRNA gene for *Chlamydia trachomatis* the 16S rRNA gene and *porA* pseudogene for *Neisseria gonorrhoeae* the 16S rRNA gene for *Mycoplasma genitalium*
Analytical Specificity	*Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Mycoplasma genitalium*, 100 %
Analytical Sensitivity (LoD)	reaches up to 0.177 cp/µl with the probability of 95 % (on Amplirun® *Chlamydia trachomatis* DNA control, Vircell) reaches up to 0.22 cp/µl with the probability of 95 % (on Amplirun® *Neisseria gonorrhoeae* DNA control, Vircell) reaches up to 1.129 cp/µl with the probability of 95 % (on Amplirun® *Mycoplasma genitalium* DNA control, Vircell)
Diagnostic Specificity	96.89% ($CI_{95\%}$: 93.04% - 98.73%)
Diagnostic Sensitivity	97.67% ($CI_{95\%}$: 86.20% - 99.88%)
Validated Specimen	swab, urine
External Quality Assessment	regularly tested by QCMD and Instand e.V. External Quality Assessment Panels
Regulatory Status	CE_{1023} IVD

Quality management system is certified in compliance with the requirements of the standard ČSN EN ISO 13485 ed.2:2016

INTERFERENCES

The interferences testing was performed using negative urine spiked with pathogen's positive controls at 3x LoD. The tested pathogens were *Chlamydia trachomatis, Neisseria gonorrhoeae* and *Mycoplasma genitalium*. Varying levels of bilirubin, urea, uric acid, albumin, low pH, high pH level and D-glucose in samples have been tested in the presence and absence of pathogen's DNA. The pathological levels of all markers for testing have been set according to the literature, hospital recommendations and guidelines (http://www.southend.nhs.uk/media/180421/pf_biochemistry_reference_intervals.pdf) and recommendations of Czech Society of Clinical Biochemistry.

URINE

Tested substance	Tested level(s)	Observed interference	Tested substance	Tested level(s)	Observed interference
Albumin	5 %	None	pH	Basic condition (pH 9)	Partial
Bilirubin	1 % (w/v)	None	Urea	300 mM; 600 mM	Partial
Glucose	0.1% (w/v); 1% (w/v)	Partial	Uric acid	5 mmol/L	Partial
pH	Acidic condition (pH 4)	Partial			

The results indicate no interference with respect to CNMX assay sensitivity, however, the partial inhibition was recognized based on missing of an internal standard. There is no risk for false negative results. There is the possibility of increased number of invalid results in terms of inhibition. The interfering endogenous substances were tested for routine concentration level in cervical swabs during the clinical validation study. There were no significant interferences observed in a set of clinical samples in respect to sensitivity and validity of examination.

The interfering endogenous substances were tested for routine concentration level in cervical swabs. According to the CLSI EP07-A2 and CLSI MM3-A3 the proposed interferent substances are blood in the concentration of 2 %, mucin in the concentration 60 µl/mL and human DNA in concentration 1-2000 µg/L. Blood was reported as an inhibitor if it was present at 4 % or more of the reaction volume (≥4 µl of blood/100 µl reaction mixture). It is recommended keeping blood below 1 to 2 % of the 100 µl reaction volume to enable amplification of sequences in blood (Kern et al., 2009). A Heme, blood compound copurified with deoxyribonucleic acid (DNA) was described as a major inhibitor of PCR amplification from bloodstains. Glycoproteins and complex polysacharides were also described as a potent PCR inhibitors (Schrader et al., 2012).

CLSI EP07-A2 - Clinical and Laboratory Standards Institute (CLSI) (2004) Interference Testing in Chemistry; Approved Guideline. CLSI document EP07-A2. CLSI. 940 West Valley Road; Suite 1400, Wayne; Pennsylvania 19087-1898 USA.

CLSI MM3-A3 - Clinical and Laboratory Standards Institute (CLSI) (2015) Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline. CLSI document MM3-A3. CLSI. 940 West Valley Road; Suite 1400, Wayne; Pennsylvania 19087-1898 USA.

Kern, M., S. Böhm, L. Deml, H. Wolf, U. Reischl, and H. H. Niller. 2009. Inhibition of Legionella pneumophila PCR in respiratory samples: A quantitative approach. Journal of Microbiological Methods 79:189-193

Schrader C, Schielke A, Ellerbroek L, Johne R. PCR inhibitors - occurrence, properties and removal. J Appl Microbiol. 2012 Nov;113(5):1014-26. doi:10.1111/j.1365-2672.2012.05384.x. Epub 2012 Jul 24. Review. PubMed PMID: 22747964.

METHOD PRINCIPLE

The PCR kit is designed for *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Mycoplasma genitalium* detection by the real-time Polymerase Chain Reaction (PCR) method. The *C. trachomatis* detection consists in amplification of both the cryptic plasmid multi-copy sequence and the 16S rRNA gene specific for *C. trachomatis* and in measurement of fluorescence increase. The *N. gonorrhoeae* detection consists in amplification of multi-copy sequence of the gene encoding 16S rRNA and porA pseudogene and in measurement of fluorescence increase. The *M. genitalium* detection consists in amplification of a multicopy sequence of the gene encoding the 16S rRNA and in measurement of fluorescence increase. The *C. trachomatis* presence is indicated by the FAM fluorophore, *N. gonorrhoeae* by Cy5 fluorophore and *M. genitalium* by TexasRed fluorophore fluorescence growth. An Internal Standard (IS) is either included in the reaction mix, controlling the possible inhibition of the PCR (ISIN version) or excluded, controlling also the DNA extraction process quality (ISEX version). IS positive amplification is detected in the HEX fluorophore fluorescence channel. The detection kit takes advantage of the "hot start" technology, minimizing non-specific reactions and assuring maximum sensitivity. Ready to Use MasterMix contains uracil-DNA glycosylase (UDG), eliminating possible contamination of the PCR by amplification products. The kit is designed for *in vitro* diagnostics and provides qualitative detection.

ISIN version

Internal Standard is included in the MasterMix tube. This PCR kit version enables PCR inhibition control.

ISEX version

Internal Standard is provided as independent item within the package. This PCR kit version enables both PCR inhibition control and nucleic acid purificaion process efficiency control.

USER MANUAL

SAMPLING AND SAMPLE STORAGE

In case of long-term storage, extracted DNA should be kept at -20 ± 5 °C to minimize degradative activity of DNases. DNA should be stored in tightly capped, hydrophobic, plastic tube (polypropylene), preferably with a rubber gasket to prevent evaporation. Extracted DNA can be stored safely in TE (Tris-EDTA, pH of 7.2) buffer at laboratory temperature (20 ± 5 °C) for 26 weeks, at 2 - 8 °C for at least one year if contaminating DNases are absent, and for up to seven years at -20 °C and at least seven years at -70 °C or lower. Samples of questionable purity should be stored at or below -20 °C to ensure DNA integrity. Repetitive freeze-thaw cycles result in the degradation of DNA.

NUCLEIC ACID PURIFICATION

Nucleic acid extraction should be performed by extraction kits available at the market according to protocols for the particular clinical material extraction. The manufacturer recommends the following extraction kits:

croBEE NA16 Nucleic Acid Extraction System
GeneProof PathogenFree DNA Isolation Kit

When using the ISEX versions of the PCR kits the IS should be added directly into the sample at the beginning of the isolation process so that in the end 1 µl of the resulting elution volume contains 0.1 µl of the IS:

Elution volume	**25 µl**	**50 µl**	**100 µl**	**200 µl**
Internal Standard	2,5 µl	5 µl	10 µl	20 µl

PCR SETUP

1. Add 30 µl of MasterMix into PCR tubes.

2. Add 10 µl of the isolated nucleic acid sample or 10 µl of Positive Control into the individual PCR tubes. The final reaction mix volume will be 40 µl. It is necessary to keep all components at +2 °C to +8 °C during the PCR preparation. The customer has to use his own negative control in the form of water, buffer or isolate of negative clinical material in each test.

3. Close the tubes, centrifuge shortly, insert them into the device and let them amplify according to the following PCR profile.
Be very careful when handling the Positive Control or the clinical material, incorrect handling could result in contamination and the consequent impairment of the kit components! The manufacturer is not responsible for the kit impairment due to incorrect handling.

AMPLIFICATION PROFILE

Step	Temperature	Time	Data Collection	Cycles
Hold	37 °C	2 min		1
Hold	95 °C	10 min		1
PCR	95 °C	5 s	FAM+HEX+Cy5+TexRed	45
	60 °C	40 s		
	72 °C	20 s		

INSTRUMENTS

GeneProof CT/NG/MG Multiplex PCR Kit is designed for use with real-time devices from various manufacturers:

	CHT	**IC**	**NG**	**MG**
croBEE Real-Time PCR System	FAM	HEX	Cy5	TexRed
Applied Biosystems 7500 Real-Time PCR System	FAM	JOE	Cy5	TexRed
CFX96™/Dx Real-Time PCR Detection System	FAM	HEX	Cy5	TexRed
LineGene 9600 Plus	FAM	HEX	Cy5	TexRed
Mic qPCR Cycler	FAM	HEX	Cy5	TexRed
QuantStudio™ 5 Real-Time PCR System	FAM	VIC	Cy5	ROX
Rotor-Gene 3000 / Q	FAM	JOE	Cy5	ROX

Required channels: FAM, HEX, Cy5, TexRed
GeneProof diagnostic kits are continually verified with various types of devices. Current list is available at www.geneproof.com or request the list at support@geneproof.com.

CLINICAL SAMPLE ANALYSIS EVALUATION

FAM	Cy5	TEX/TexRed/ ROX	HEX/JOE/VIC	Interpretation
+	+	+	+/-	*C. trachomatis, N. gonorrhoeae and M. genitalium positive*
+	-	+	+/-	*C. trachomatis and M. genitalium positive*
+	+	-	+/-	*C. trachomatis and N. gonorrhoeae positive*
+	-	-	+/-	*Chlamydia trachomatis positive*
-	+	+	+/-	*N. gonorrhoeae and M. genitalium positive*
-	-	+	+/-	*Mycoplasma genitalium positive*
-	+	-	+/-	*Neisseria gonorrhoeae positive*
-	-	-	+ (Ct < 38)	*Negative*
-	-	-	-	*Invalid*
-	-	-	+ (Ct > 38)	*Invalid*

WARNING

A single valid Instruction for use for a specific kit is included in the package or to be requested for the particular lot from the manufacturer. The kit should be disposed of after use according to the current legal regulations considering the fact that the kit does not contain any dangerous, infectious or toxic components that would be subject to special safety regulations, and the packaging materials are made of paper and polypropylene. If you have any questions please contact our Customer Service.

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