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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Clonit'*nGo* STD Top 7

For the specific identification and differentiation of Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma genitalium, Trichomonas vaginalis, Ureaplasma urealyticum, Ureaplasma parvum and Mycoplasma hominis, by *Real Time PCR.*

IVD	***In vitro* diagnostic device**
	Revision 3 –15th November 2022
	Range of temperature
	Use within (dd/mm/yyyy: year-month)
LOT	Lot (xxxx)
REF	CLNG-48-00
	CLONIT srl Via Umberto Saba 25 - 20081 Abbiategrasso (MI) Via Varese 20 – 20121 Milano (MI)
	48 Tests

INTRODUCTION AND PURPOSE OF USE

Clonit'nGoSTD Top 7 is a real-time PCR detection kit for the identification and specific differentiation of Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma genitalium, Trichomonas vaginalis, Ureaplasma urealyticum, Ureaplasma parvum and / or Mycoplasma hominis, in specimens patients with signs and symptoms of sexually transmitted diseases (STDs). After DNA isolation, STD identification is performed by amplification of a conserved region of the T. vaginalis specific 2-kb repeat sequence (Trichomonas vaginalis), of the urease gene (Ureaplasma urealyticum and Ureaplasma parvum), yidC gene (Mycoplasma hominis), porA and Opa (Neisseria gonorrhoeae), a region within ORF2 of the chlamydial plasmid gene (Chlamydia trachomatis) and MgPa adhesin (Mycoplasma genitalium), using specific primers and a fluorescently labeled probe.

CONTENT

The kit contains reagents enough to perform 48 amplification tests

	Quantity	Description
R1	6 x 8 well strips	Neisseria gonorrhoeae, Chlamydia trachomatis & Mycoplasma genitalium
R2	6 x 8 well strips	Trichomonas vaginalis, Ureaplasma urealyticum, Ureaplasma parvum & Mycoplasma hominis
R3	1 vial	STD – Positive Control
R4	1 vial x 1ml	Water RNAse/DNAse free (Buffer A)
R5	1 vial x 1.8ml	Rehydratation Buffer (Buffer B)
R6	1 vial x 1ml	Negative control (Buffer C)
	12	8-cap strips

Instruction for use: **ST. CLNG-4800.ENG.4**

MATERIALS AND STRUMENTATION REQUIRED BUT NOT SUPPLIED

The following list includes the materials that are required for use but not included in Clonit'nGoSTD Top 7 Real Time PCR Detection Kit.
Real Time PCR instrument (thermocycler).
DNA extraction kit.
Centrifuge for 1.5 mL tubes and PCR-well strips or 96-well plate (if available).
Vortex. F
Micropipettes (0.5-20 µL, 20-200 µL)
Filter tips.
Powder-free disposable gloves.
Loading block (for use with Qiagen/Corbett Rotor-Gene® instruments).

INSTRUMENT

Clonit'nGoSTD Top 7 Kit has been validated on the following equipments:
Applied Biosystems 7500 Fast Real-Time PCR System,
Bio-Rad CFX96™ Real-Time PCR Detection System,
Agilent Technologies AriaMx Real-Time PCR System,
DNA-Technology DTprime Real-time Detection Thermal Cycler,

DNA-Technology DTlite Real-Time PCR System,
Rotor-Gene® Q (Qiagen),
SmartCycler® (Cepheid)
Roche Molecular Diagnostics Cobas z480 Analyzer.

When using the Applied Biosystems 7500 Fast with strips it is recommend to place a plate holder to reduce the risk of crushed tube (Ref. PN 4388506).

Please ensure that the instruments have been installed, calibrated, checked and maintained according to the manufacturer's instruction and recommendations

SAMPLE AND STORAGE

Clinical specimens (urogenital specimens, urine samples, rectal and endocervical samples) should be collected in clean containers and processed as soon as possible to guarantee the quality of the test. We recommend to use fresh samples. For longer storage, the samples should be frozen at -20ºC. In this case, the sample will be totally thawed and brought to room temperature before testing. Homogenize sample as thoroughly as possible prior to preparation. Freezing and thawing cycles are not recommended. Perform the sample preparation according to the recommendations appearing in the instructions for use of the extraction kit used.

PREACAUTION USE

The product is indented for use by professional users only, such as laboratory or health professionals and technicians, trained in molecular biological techniques.
Do not use past expiration date.
Do not use reagents if the protective pouches are open or broken upon arrival.
Do not use reagents if desiccant is not present or broken inside reagent pouches.
Do not remove desiccant from reagent pouches once is open.
Close protective pouches of reagents promptly with the zip seal after each use
Remove any excess air in the pouches prior to sealing.
Do not use reagents if the foil has been broken or damaged.
Do not mix reagents from different envelopes and / or kits and / or lots and / or another supplier.
Protect reagents against from humidity. Prolonged exposure to humidity may affect product performance.
Make sure to use a well for determining Neisseria gonorrhoeae, Chlamydia trachomatis and Mycoplasma genitalium and another well for determining Trichomonas vaginalis, Ureaplasma urealyticum, Ureaplasma parvum and Mycoplasma hominis. Be careful not to mix them throughout the process.

LIMIT OF THE METHOD

The results of the test should be evaluated by a health care professional in the context of medical history, clinical symptoms and other diagnostic tests.

Although this assay can be used with other types of samples it has been validated only with DNA extracted from urogenital specimens and endocervical specimens for Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma genitalium, Trichomonas vaginalis, Ureaplasma urealyticum, Ureaplasma parvum and Mycoplasma hominis, rectal specimens (rectal-vaginal specimens and rectal samples) for Neisseria gonorrhoeae, Ureaplasma urealyticum and Mycoplasma hominis; and urine samples for Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma genitalium, Ureaplasma urealyticum, Ureaplasma parvum and Mycoplasma hominis.

ON only intervenes in the conformity assessment of the test for Chlamydia trachomatis. The scope of the CE certification covers the detection of Chlamydia trachomatis from urine samples and urogenital and endocervical swabs. The detection of Chlamydia trachomatis in pharyngeal, semen, serum and

rectal, urethral and vaginal swabs are out of the scope of certification by ON. The rest of the pathogens have self-certification for CE marking.

The quality of the test depends on the quality of the sample; proper extracted nucleic acid from clinical samples must be extracted. Unsuitable collection, storage and/or transport of specimens may give false negative results.

Extremely low levels of target below the limit of detection might be detected, but results may not be reproducible.

There is a possibility of false positive results due to cross-contamination by contamination by Sexually Transmitted Diseases, either samples containing high concentrations of target DNA or contamination due to PCR products from previous reactions.

STORAGE AND STABILITY

The kits can be shipped and stored at 2 a 40 °C until the expiration date which is stated on the label. Once the positive control has been re-suspended, store it at -20ºC. We recommend to separate it in aliquots to minimize freeze and thaw cycles. Keep components away from sunlight.

ANALYTICAL PROCEDURE

Clinical specimens (urogenital specimens, urine samples, rectal and endocervical samples) should be collected in clean containers and processed as soon as possible to guarantee the quality of the test. We recommend to use fresh samples. For longer storage, the samples should be frozen at -20ºC. In this case, the sample will be totally thawed and brought to room temperature before testing. Homogenize sample as thoroughly as possible prior to preparation. Freezing and thawing cycles are not recommended. Perform the sample preparation according to the recommendations appearing in the instructions for use of the extraction kit used.
Perform the sample preparation according to the recommendations appearing in the instructions for use of the extraction kit used. For DNA extraction from clinical samples you can use your manual or automatic routine optimized system. Also, you can use any commercially available DNA extraction kit and follow the manufacturer´s instructions.
Maxwell® 16 Viral Total Nucleic Acid Purification Kit, using the Maxwell® 16 instrument (Promega).
ZP02006 MagPurix Bacterial DNA Extraction Kit, using the MagPurix 12A instrument (Zinexts Life Science Corp.).
US121 NX-48 Urine/Swab DNA Kit using the Nextractor® NX-48 (Genolution Inc.).

POSITIVE CONTROL

STD Top 7 Positive control contains high copies of the template, the recommendation is to open and manipulate it in a separate laboratory area away from the other components. Reconstitute the lyophilized Sexually Transmitted Diseases Positive Control (red vial) by adding 200 µL of the supplied Water RNAse/DNAse free (white vial) and vortex thoroughly.

PRECEDURE

Determine and separate the number of required reactions including samples and controls. One positive and negative control must be included in each run for each assay.
Peel off protective aluminium seal from plates or strips.
Reconstitute the number of wells you need. Add 15 µL of Rehydration Buffer (blue vial) into each well.
Adding samples and controls. Add 5 µL of DNA extracted from each sample, reconstituted Sexually Transmitted Diseases Positive Control or Negative Control in different wells and close them with the provided caps.

It is recommended to briefly centrifuge the 8-well strips, or gently tap each strip onto a hard surface to ensure that all the liquids are at the bottom of the tubes. Load the plate or the strips in the thermocycler.

SOFTWARE SETTINGS

Set up the thermocycler. Program the thermocycler following the conditions listed below and start the run:

Cycles	***Denaturation***		***Annealing/ extension***	
1	*95°C 2 min*		*Reading stage*	
45	*95°C*	*10 sec*	*60°C*	*50 sec*

Fluorogenic data should be collected during the extension step.

R1	***Internal Control***	***C.trachomatis***	***N.gonorrhoeae***	***M. genitalium***
7500 Lifetech.	*Cy5*	*FAM*	*ROX*	*VIC*
CFX 96	*Cy5*	*FAM*	*ROX*	*HEX/JOE*
RotorGene Q	Red	*Green*	*Orange*	*Yellow*
QS5 *Lifetech.*	*Cy5*	*FAM*	*ROX*	*VIC*

R2	**M.hominis**	***T.vaginalis***	***U.parvum***	***U.urealyticum***
7500 Lifetech.	*Cy5*	*FAM*	*ROX*	*VIC*
CFX 96	*Cy5*	*FAM*	*ROX*	*HEX/JOE*
RotorGene Q	Red	*Green*	*Orange*	*Yellow*
QS5 *Lifetech.*	*Cy5*	*FAM*	*ROX*	*VIC*

In Applied Biosystems 7500 Fast Real-Time PCR System and Stratagene Mx3005P™ Real Time PCR System check that passive reference option ROX is none. In the Applied Biosystems 7500 Fast Real-Time PCR System select Ramp Speed Standard in Select New Experiment/Advanced Setup/Experiment Properties.

Reaction volume: 20 µl.

RESULTS INTERPRETATION

The use of positive and negative controls in each run, validate the reaction by checking the absence of signal in negative control well and the presence of signal for Sexually Transmitted Diseases Positive Control well. The analysis of the samples is done by the software itself of the used real time PCR equipment according to manufacturer´s instructions.

Interpretation of results for Neisseria gonorrhoeae, Chlamydia trachomatis & Mycoplasma genitalium 8-well strips:

- A sample is considered positive for Chlamydia trachomatis if there is an amplification signal in FAM channel, the Ct value obtained is less than 40 and the internal control shows or not an amplification signal. Sometimes, the detection of internal control is not necessary because a high copy number of target can cause preferential amplification of target-specific nucleic acids.
- A sample is considered positive for Neisseria gonorrhoeae if there is an amplification signal in ROX channel, the Ct value obtained is less than 40 and the internal control shows or not an amplification signal. Sometimes, the detection of internal control is not necessary because a high copy number of target can cause preferential amplification of target-specific nucleic acids

- A sample is considered positive for Mycoplasma genitalium if there is an amplification signal in HEX channel, the Ct value obtained is less than 40 and the internal control shows or not an amplification signal. Sometimes, the detection of internal control is not necessary because a high copy number of target can cause preferential amplification of target-specific nucleic acids.
- A sample is considered negative, if the sample shows no amplification signal in the detection system but the internal control is positive. - The experiment is considered failed if there is an amplification signal in the Negative Control well and/or there is not amplification signal in the Positive Control well. We recommend to repeat the assay again.
- In case of absence of internal control signal in sample wells we recommend to repeat the assay diluting the sample 1:10 or to repeat the extraction to check for possible problems of inhibition.

Interpretation of results for Trichomonas vaginalis, Ureaplasma urealyticum, Ureaplasma parvum & Mycoplasma hominis 4/8-well strips:

- A sample is considered positive for Trichomonas vaginalis if there is an amplification signal in FAM channel and the Ct value obtained is less than 40.
- A sample is considered positive for Ureaplasma urealyticum if there is an amplification signal in HEX/VIC/JOE channel and the Ct value obtained is less than 40.
- A sample is considered positive for Ureaplasma parvum if there is an amplification signal in ROX channel and the Ct value obtained is less than 40.
- A sample is considered positive for Mycoplasma hominis if there is an amplification signal in Cy5 channel and the Ct value obtained is less than 40. - A sample is considered negative, if the sample shows no amplification signal in the detection system.
- The experiment is considered failed if there is an amplification signal in the Negative Control well and/or there is not amplification signal in the Positive Control well. We recommend to repeat the assay again.

PERFORMACES EVALUATION

Clinical sensitivity and specificity

The clinical performance of Clonit'nGoSTD Top 7 Kit was tested using 948 different specimens first-void urines, rectal swabs, urethral swabs, endocervical swabs/exudate, vaginal swabs and pharyngeal swabs from symptomatic patients. Of all the analyzed samples, 46 discordant samples were obtained. These discrepancies were mainly observed in samples close to the limit of detection.

These results of both Multiplex reactions were compared with those obtained with a molecular detection method (AllplexTM STI Essential Assay (Seegene)). Of the 46 discordant samples, it was possible to solve the discrepancy using FTD Urethritis plus (Fast Track Diagnostics) in 35 samples, the rest could not be re-analysed for lack of sample.

The results were as follows

Clonit'nGoSTD Top 7 Neisseria gonorrhoeae	Allplex STI Essential Assay (seegene)			
		+	-	Total
	+	19	1	20
	-	1	927	928
	Total	20	928	948

Clonit'nGoSTD Top 7 C. trachomatis	Allplex STI Essential Assay (seegene)			
		+	-	Total
	+	68	3	71
	-	2	875	877

	Total	70	878	948

Clonit´nGoSTD Top 7 M.genitalium	Allplex STI Essential Assay (seegene)			
		+	-	Total
	+	28	1	29
	-	2	917	918
	Total	30	918	948

Clonit´nGoSTD Top 7 T.vaginalis	Allplex STI Essential Assay (seegene)			
		+	-	Total
	+	20	3	23
	-	0	925	925
	Total	20	928	948

Clonit´nGoSTD Top 7 U.urealyticum	Allplex STI Essential Assay (seegene)			
		+	-	Total
	+	166	6	172
	-	7	769	776
	Total	173	775	948

Clonit´nGoSTD Top 7 U.parvum	Allplex STI Essential Assay (seegene)			
		+	-	Total
	+	402	13	415
	-	5	528	533
	Total	407	541	948

Clonit´nGoSTD Top 7 M.hominis	Allplex STI Essential Assay (seegene)			
		+	-	Total
	+	183	2	185
	-	0	763	763
	Total	183	765	948

Sensitivity, specificity, PPV and NPV values for Clonit´nGoSTD Top 7 (CLONIT) compared with AllplexTM STI Essential Assay (Seegene) are shown in next table:

Target	SE(%)	SP(%)	VPP(%)	VPN (%)
C.trachomatis	97.14	99.6	95.75	99.77
M.genitalium	93.33	99.89	96.55	99.78
N.gonorrhoeae	95	99.89	95	99.79
T.vaginalis	100	99.68	89.96	100
U.urealyticum	95.95	99.23	96.51	99.1
U.parvum	98.77	97.6	96.87	99.06
M.homins	100	99.74	98.92	100

Analytical sensitivity:
It is considered as analytical sensitivity the highest dilution (title) to which a positive sample can be diluted without the system losing the ability to detect as positive.
Clonit´nGoSTD Top 7 has a detection limit of ≥10 DNA copies per reaction for each target.

Analytical specificity:
The specificity of the Clonit'nGoSTD Top 7 assay was confirmed by testing a panel consisting of different microorganisms representing the most common Sexually Transmitted Diseases pathogens. No cross-reactivity was detected against any of the following microorganisms tested:

Samples	Results	Samples	Results
Candida parapsilosis	-	Acinetobacter baumannii	-
Escherichia Coli	-	Listeria Innocua	-
Serratia marcescens	-	Ureaplasma urealyticum	-/+
Candida tropicalis	-	Chlamydia trachomatis (LGV)	-/+
Gardnerella vaginalis	-	Mycoplasma genitalium	-/+
Staphylococcus aureu	-	Cytomegalovirus	-
Candida glabrata	-	Chlamydia trachomatis (SW)	-/+
Haemophilus influenzae	-	Mycoplasma hominis	-/+
Stenotrophomonas maltophilia	-	Herpes simplex virus 1	-
Candida krusei	-	Enterobacter cloacae	-
Haemophilus ducreyi class 1	-	Neisseria gonorrhoeae	-/+
Streptococcus agalactiae	-/+	Herpes simplex virus 2	-
Candida dubliniensis	-	Enterobacter aerogenes	-
Klebsiella oxytoca	-	Neisseria meningitidis	-
Streptococcus pneumoniae	-	Human papillomavirus 16	-
Candida albicans	-	Enterococcus faecalis	-
Klebsiella pneumoniae	-	Proteus mirabilis	-
Treponema pallidum	-	Human papillomavirus 18	-
Aspergillus fumigatus	-	Enterococcus faecium	-
Listeria Ivanovii	-	Pseudomonas aeruginosa	-
Trichomonas vaginalis	-	Hepatitis A	-
Bacteroides fragilis	-		
Listeria monocytogenes	-		
Ureaplasma parvum	-/+		

INTERFERENCES

Verify that in the DNA extracted from the sample there is no contamination from mucoproteins and haemoglobin, to exclude possible inhibition of PCR reaction. The interference due to contaminants can be detected through a spectrophotometric analysis, verifying the ratio between the absorbance readings at 260 nm (maximum absorbtion of Nucleic Acids) and 280 nm (maximum absorbtion of Proteins). A pure DNA should have a ratio of approximately 1.8

QUALITY CONTROL

It is recommended to include in each analytical run, as quality control of every extraction, amplification and detection step, an already tested negative and positive sample, or a reference material with known concentration
In accordance with the Clonit srl ISO EN 13485 Certified quality Management System, each lot of Clonit'nGoSTD Top 7 is tested against predetermined specification to ensure consistent product quality.

TECHNICAL ASSISTANCE

For any question and support please contact our Technical support:

e-mail: info@clonit.it
phone: +39 02 56814413

IVD	*In vitro* diagnostic device
	Read the instruction's manual
	Range of temperature
	Use within (dd/mm/yyyy: year-month)
LOT	Lot (xxxx)
REF	Code
	Manufacturer
	Contains sufficient for <n> tests

Clonit'^{nGo}STD Top 7 is CE marked diagnostic kit according to the European *in vitro* diagnostic directive 98/79/CE. ON only intervenes in the conformity assessment of the test for Chlamydia trachomatis. The scope of the CE certification covers the detection of Chlamydia trachomatis from urine samples and urogenital and endocervical swabs. The detection of Chlamydia trachomatis in pharyngeal, semen, serum and rectal, urethral and vaginal swabs are out of the scope of certification by ON. The rest of the pathogens have self-certification for CE marking.

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CE IVD

for *in vitro* diagnostic use