CE 0318*

STI CNM Real Time PCR Kit

Kit for the detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Mycoplasma genitalium* by Real-Time PCR

REF **Ref. MAD-003948M-W** Σ **100 determinations**

For in vitro diagnostic use only*
Directive 98/79/EC

**The Notified Body 0318 only intervenes in the evaluation of the compliance of the test for Chlamydia trachomatis in urine and semen; urethral, endocervical and anal swabs. The rest of pathogens have the self-certified CE marking.*

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1 INTENDED USE

STI CNM Real Time PCR Kit is an in vitro diagnostic kit for the qualitative detection of the DNA of pathogenic organisms causing sexually transmitted diseases (STD) in humans such as *Chlamydia trachomatis*, Neisseria gonorrhoeae* and *Mycoplasma genitalium*, from DNA extracted from human clinical samples of different origin such as urine and semen; urethral, endocervical, anal and pharyngeal swabs. It is based on the multiplex real-time PCR technique, using primers and fluorescent probes for the cryptic plasmid target genes, OpaI and MgPa of *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Mycoplasma genitalium*, respectively.

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Specific primers and fluorescent probe are also included for the simultaneous detection of the human RNaseP gene as internal quality control of the starting and amplification material. The detection channels of the different targets are:

Target	Fluorophore
Chlamydia trachomatis	ROX
Neisseria gonorrhoeae	FAM
Mycoplasma genitalium	JOE
RNaseP	Cy5

Table 1. Detection channels for the different targets of STI CNM Real Time PCR kit.

This test must be performed at hospital level in clinical microbiology laboratories to those patients who show symptoms compatible with a sexually transmitted infection. The intended end use is as aid in the diagnosis of sexually transmitted diseases in combination with clinical risk and epidemiological factors.

Microbiological status: Non-sterile product.

2 PRINCIPLE OF THE METHOD

STI CNM Real Time PCR kit is a multiplex assay based on the real-time polymerase chain reaction. The Master mix contains three sets of primers and probes for the detection of bacterial DNA of *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Mycoplasma genitalium*. It also includes primers and probes for the detection of the human gene of the RNaseP in clinical or control samples. The oligonucleotides used as primers and probes were selected in evolutionary conserved regions.

In the presence of any of these bacteria in clinical samples, bacterial DNA is amplified by polymerase chain reaction (PCR). The detection of amplicons is based on the use of double-labeled probes with a reporter-quencher combination that allows hybridization of the probe with the double helix DNA molecules produced in each amplification cycle and hydrolysis of the probe in successive cycles. This way, while the extension phase of the PCR occurs, the 5' nuclease activity of the polymerase DNA degrades the probes bound specifically to their targets, causing the split between the reporter and the quencher, and a fluorescent signal will be

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generated. The specific probes for each bacterium will generate a fluorescent signal at different wavelenghts, allowing the Real Time PCR instrument to differentiate between the different signals. In each denaturation-extension cycle, the split of new molecules of reporter occurs, and, consequently, the intensity of the fluorescent signal increases. The intensity of the fluorescence is monitored on the real-time PCR instruments in each of the cycles and the data are analyzed with a specific analysis software for each platform.

The detection of bacterial DNA is of great usefulness in the diagnosis and monitoring of infections caused by these microorganisms.

3 COMPONENTS

STI CNM Time PCR kit is commercialized as a ready-to-use Master Mix which includes all the necessary reagents to perform the real-time PCR.

Furthermore, in order to avoid contamination with previous PCR products, the Mix contains the enzyme Uracil-DNA Glycosylase (Cod-UNG), which degrades PCR products containing dUTP.

A positive control (PC) and DNase/RNase-free DEPC-treated water to include in the negative controls (NTC) are supplied along with the PCR Mix.

Components of the kit for 100 tests:

REFERENCE (DESCRIPTION)		CONTENT	AMOUNT
MAD-003948M-100-W (STI CNM MMIX)	**MAD-003948-MIX-W (STI CNM MMix)**	Hot Start Polymerase (125 U/ml), Uracil DNA glycosylase (50 U/mL), 0.1-0.4 µM primers, 0.05-0.4 fluorescent probes, 2x reaction buffer, 1 mM dUTP, 1.3 mM dNTPs (A, G, C, T)	**2 vials with 50 test/vial**
	MAD-DDW (RNase/DNase-free water)	---	**1 vial (200 µl)**
MAD-STI-CNM (STI CNM PC)		Non-infectious synthetic DNA containing part of the genome of *Chlamydia trachomatis, Nesseria gonorrhoeae and Mycoplasma genitalium* (12.5 copies/ml) and human DNA (0.625 ng/µl)	**1 vial (100 µl)**

Table 2. Reagents and concentrations of the active substances supplied in the STI CNM Time PCR kit in wet format.

4 ADDITIONAL REQUIRED MATERIAL NOT SUPPLIED

4.1 Reagents and materials

- Disposable gloves.
- DNase/RNase-free filtering pipette tips.
- DNA extraction kit.
- Tube strips/plates/optical adhesive films specific for each equipment of Real-Time PCR.

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4.2 Equipment

- Laminar flow cabinet
- Microcentrifuge for tubes of 1.5ml.
- Microcentrifuges of PCR tube strips or 96-well plates.
- Vortex.
- Automatic micropipettes: P1000, P200, P20 and P2.
- Real-Time PCR instrument.

5 STORAGE AND STABILITY CONDITIONS

STI CNM Real Time PCR Kit must be transported and stored between -10 and -30 °C*. However, transport at refrigerated temperature (2 °C - 8 °C) is also possible as long as the transit time does not exceed a maximum of 10 days and the kit is stored at a temperature between -10 and -30 °C upon receipt.

STI CNM MMix reaction mix:

- Is sensitive to physical state changes and it has been proven that it supports up to seven freeze/thaw cycles. If a run is performed with a low number of samples, it is recommended to aliquot the reagent in advance.
- Is stable for at least 8 months stored between -10 and -30 °C.
- Contains fluorescent molecules and must be kept away from direct light.

Positive control:

- Is sensitive to physical state changes and must not undergo more than 8 freeze/thaw cycles.
- Is stable for at least 18 months between -10 and -30 °C.
- It is advisable to handle separately from the clinical samples to avoid potential contamination which might yield false positives.

If stored at recommended temperature, the PCR reagents are stable until the expiration date indicated on the product label. The PCR reagents must be stored in areas free of DNA or PCR products contamination.

*A temperature indicator is included in the package to control the conditions during the shipment. In case the cold chain is interrupted, it is recommended to contact the manufacturer before using the reagents.

6 WARNINGS AND PRECAUTIONS

- Read the instructions for use before using this product.
- The kit must be handled by qualified technicians in molecular biology techniques applied to diagnosis.
- Do not use any component of the kit after the expiration date.

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- The STI-CNM MMix must be thawed before use and handled on ice or cold plate and away from light. Mix the solutions by inverting the tubes several times without shaking in vortex, and centrifuge briefly.
- The positive control must be thawed at room temperature, mixed well and centrifuged briefly before use.
- The safety and disposal precautions are described in the Safety Data Sheet of this product. This product is only intended for professional laboratory purposes, and it is not intended for pharmacological, home or any other type of use. The current version of the Safety Data Sheet of this product can be downloaded in the web page www.vitro.bio or requested at regulatory@vitro.bio.
- STI CNM **Real Time PCR** kit uses nucleic acids previously extracted and purified as starting material. It is the client's responsibility to include the necessary controls to verify that the system of extraction of the used genetic material works properly.

- **General considerations to avoid the contamination with PCR product**

  The most important contamination source is usually the same amplified PCR product. Therefore, it is recommended to carry out the amplification and handling of the amplified products in a different area to the one where the DNA extraction and PCR preparation are performed. It is recommended to work in different pre- and post-PCR areas where the handling of the test DNA and preparation of the PCR tubes (pre-PCR), and the amplification and handling of the amplified products (post-PCR) are performed. These areas must be physically separated, and different laboratory material must be used (laboratory coats, pipettes, tips, etc.) to avoid the contamination of the samples with the amplified DNA, which could lead to false positive diagnoses. The workflow must always go in a single direction, from the pre-PCR area to the post-PCR area and never the opposite way. The material and personal flow from the post-PCR area to the pre-PCR area must be avoided. Furthermore, in order to avoid the contamination with previous PCR products, the enzyme *Uracil-DNA Glycosylase (Cod-UNG)*, which degrades the PCR products containing dUTP, is included in the kit.

  It is recommended to include negative amplification controls replacing the DNA specimen with RNase/DNase-free water in order to detect and control any possible contamination of the reagents with test samples or amplified products.

- **Waste disposal**

  The handling of wastes generated by the use of the products commercialized by Vitro S.A. must be performed according to the applicable law in the country in which these products are being used. As reference, the following table indicates the classification of waste generated by this kit according to the European Law, specifically according to the European Commission Decision of December 18, 2014 amending decision 2000/532/EC on the list of waste pursuant to Directive 2008/98/EC of the European Parliament and of the Council:

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POTENTIAL WASTE GENERATED AFTER USING THIS PRODUCT	ELW CODE*	TYPE OF WASTE ACCORDING TO ELW*
1. Liquid waste disposal	161001	"Aqueous liquid wastes containing dangerous substances" after adding 10% of the total volume of a disinfectant agent. If the disinfection is not carried out, this waste must be considered as "waste whose storage and disposal is subjected to special requirements in order to prevent infection"
2. Perishable material (tubes, tips, etc.) 3. Any element that has been in contact with the starting genetic material	180103	"Waste whose collection and disposal is subject to special requirements in order to prevent infection"
4. Container for reagents used classified as dangerous (according to the Safety Data Sheet)	150110	"Containers containing waste or contaminated by dangerous substances"

Table 3. Classification of wastes generated by this kit according to the European Legislation. *ELW: *European Legislation of Waste.*

*Note: This classification is included as a general guideline of action, being under the final responsibility of the user the accomplishment of all the local, regional and national regulations on the disposal of this type of materials.

7 PREPARATION OF THE CLINICAL SAMPLE FOR ANALYSIS

7.1 Sample collection

STI CNM Real Time PCR kit has been validated for its use starting from purified genetic material from different types of clinical samples, such as urine, semen; urethral, endocervical, rectal and pharyngeal swabs, and liquid-based endocervical cytologies.

The conditions for sample collection, handling and preparation will depend on the type of sample.

7.1.1. Cytological samples

Swabs are performed with a small swab or a sterile brush (cytobrush). The swab with the collected cells should be placed in a sterile container with a suitable transport medium (e.g., 1X PBS, physiological/isotonic solution).

Store the sample between +2 °C and +8 °C and extract the nucleic acids within a week. If extraction is not feasible within one week, store samples at -30 °C to -20 °C.

In case the number of cells in a sample is too low, centrifuge repeatedly before starting the extraction. After each centrifugation step resuspend in sterile PBS. Centrifugation/resuspension cycles can also be used to remove mucus, red blood cells or other material.

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7.1.2. Semen

Seminal fluid is collected in a sterile container and can be stored at +2 °C to +8 °C for several hours (maximum overnight). Alternatively, freeze the sample after addition of a medium that is compatible with the DNA extraction method.

7.1.3. Urine

In general, early morning urine samples are used for the detection of *Chlamydia trachomatis, Neisseria gonorrhoeae* and *Mycoplasma genitalium*. When collecting the sample, the first part of the urine should be discarded and only the second part should be collected. Use a sterilized container.

If the clinical samples are not to be processed immediately upon receipt, it is recommended to store them at 4°C for a maximum period of 1 week.

This kit has been validated with starting genetic material obtained from the following DNA/RNA purification kits and extraction kits* from 200 µl of clinical sample and eluting in 100 µl of elution buffer (for purification with Opentrons start with 92 µl of clinical specimen and elute in 60 µl of elution solution):

EXTRACTION KITS	EXTRACTION EQUIPMENT
MagNA Pure LC Total Nucleic Acid Isolation Kit I (Roche Diagnostic´s)	MagNA Pure Compact Instrument. Version 1.1.2 (Roche Diagnostic's)
Maxwell® 16 FFPE Tissue LEV ADN Purification Kit (Promega)	Maxwell® 16 (Promega)
NX48S – Urine/Swab DNA Kit (Genolution)	Nextractor NX-48S (Genolution)
RNA/DNA pathogen kit (Opentrons Robot) (Vitro, ref. MAD-003955M)	Opentrons OT-2

Table 4. Extraction kits and instruments used for the purification of DNA/RNA from clinical samples.

*Note: The system has not been validated with other DNA/RNA extraction systems. Therefore, if any other purification system is used, this must be verified beforehand.

8 PCR PROTOCOL

8.1 Preparation of the Reaction mix

The Real-Time PCR reaction is carried out in a final volume of 20 µl. Prepare the Master Mix as indicated below:

1. Thaw and homogenize STI CNM MMix (do not use vortex). Once it is thawed, centrifuge briefly.
2. Mix in each PCR tube the following volumes for each sample:

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Reagent	V/test
STI CNM MMix	12 µl
Sample	8 µl

3. Include a negative control by adding 8 µl of the water included in the kit.
4. Include a positive control by adding 8 µl of the positive DNA control STI CNM PC included in the kit.
5. Centrifuge briefly to make sure there are no air bubbles in the wells.

It is recommended to keep the MMix on cold plate during the preparation of the samples and do not thaw the vial more than five times.

8.2 Configuration of the instrument for real-time PCR

In the instrument's software enter the different targets and detection channels for each of them. Create the samples, the positive control (PC), the PCR targets (NTC) and allocate the positions of the samples in the PCR plate.

Set the real-time PCR instrument following the steps below:

PCR PROGRAM		
25°C	5 min	1 cycle
95°C	5 min	1 cycle
95°C	15 sec	45 cycles
56°C*	40 sec	

Table 5. PCR program of STI CNM Real Time PCR kit.

*The fluorescence data must be collected during the extension phase (*) by means of the FAM (NG), ROX (CT), HEX, JOE or VIC (MG) and Cy5 (internal control) channels.

This kit has been validated with the platforms:

- QuantStudio™ 5 Real-Time PCR System (Applied Biosystems)
- CFX96™ Real-Time PCR Detection System (Bio-Rad)
- VitroCycler (Vitro S.A.)

For its use in other platforms, it is recommended to verify the compatibility of the fluorochromes with the detection channels of each instrument. Although the fluorochromes included in the kit are compatible with the majority of the most-used real-time instruments available on the market. In Applied Biosystems 7500 Fast Real-Time PCR System, QuantStudio™ 5 Real-Time PCR System thermal cyclers the ROX passive control option must be disabled.

In the thermal cyclers Applied Biosystems QuantStudio™ 5 Real-Time PCR System and Applied Biosystems 7500 Fast Real-Time PCR System, select Ramp Speed Standard in the menu “Select New Experiment/Advanced Setup/Experiment Properties”.

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9 INTERPRETATION OF RESULTS

Before interpreting the results of the clinical samples, it is necessary to follow the interpretation guide of the positive and negative controls as in the table below:

	RESULT	INTERPRETATION
Positive Control STI CNM	Signal for the channels FAM, ROX, Cy5 and JOE*	The control/reaction is correct
	No signal for FAM and/or ROX and/or Cy5 and/or JOE	Problem in the amplification: repeat analysis
Negative control:	Signal for the channels FAM and/or ROX and/or Cy5 and/or JOE	Contamination, repeat analysis
	No signal	The control/reaction is correct

*The amplification signal must be determined by a rapid and steady increase in the fluorescence values and not by peak phenomena or gradual increase of the background signal (irregular background or increased background noise) (Fig 1).

The run is considered valid when adequate results have been obtained for all reaction controls and the Cts values obtained in the positive control for the different targets are within the range of expected values, being these:

- 24±2 for NG (FAM)
- 21±2 for CT (ROX)
- 23±2 for MG (JOE)
- 26±2 for RNaseP (Cy5)

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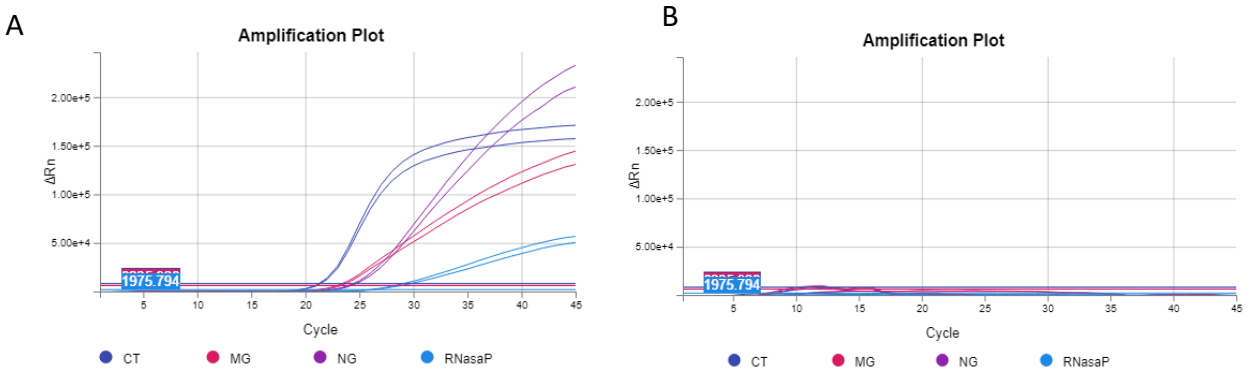

Figure 1: Graphs of amplification of the positive control PC (A) and of a negative control with water NTC (B). (Expected Cts values for PC: NG (FAM) 24±2; CT (ROX) 21±2; MG (JOE) 23±2, RNaseP (Cy5) 26±2. Experiment performed in Applied Biosystems QuantStudio™ 5 Real-Time PCR System.

If the run has been validated, interpret the results of the clinical samples according to the following table:

STI CNM Real Time PCR Kit				**INTERPRETATION**
NG (FAM)	**CT (ROX)**	**MG (JOE)**	**RNaseP (Cy5)**	
Signal	No signal	No signal	Signal	Positive sample for *Neisseria gonorrhoeae*
			No signal	
No signal	Signal	No signal	Signal	Positive sample for *Chlamydia trachomatis*
			No signal	
No signal	No signal	Signal	Signal	Positive sample for *Mycoplasma genitalium*
			No signal	
No signal	Signal	Signal	Signal	Positive sample for *Chlamydia trachomatis* and *Mycoplasma genitalium*
			No signal	
Signal	Signal	No signal	Signal	Positive sample for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*
			No signal	
Signal	No signal	Signal	Signal	Positive sample for *Neisseria gonorrhoeae* and *Mycoplasma genitalium*
			No signal	
Signal	Signal	Signal	Signal	Positive sample for *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and *Mycoplasma genitalium*
			No signal	
No signal	No signal	No signal	Signal	Negative result [(1)]
			No signal	Invalid [(2)]: Problems in the extraction or amplification

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(1) Negative or below the limit of detection of the kit.
(2) It is recommended to repeat the PCR o from a new DNA extraction.

It is recommended to use the automatic threshold adjustment made by the default software of each instrument and if necessary the threshold can be adjusted manually ensuring that it falls within the exponential phase of the fluorescence curve and that the background noise is below the threshold line.

A sample is positive if the Ct value obtained is ≤38, although the internal control does not show an amplification graph. Sometimes, it might occur that the internal control is not amplified correctly due to the presence of a high initial number of copies of target bacterial nucleic acid, which can cause a preferential amplification of the latter.

A sample is negative if an amplification curve is not detected over the threshold value, and if the internal control does show it. The inhibition of the PCR reaction can be excluded by the amplification of the internal control.

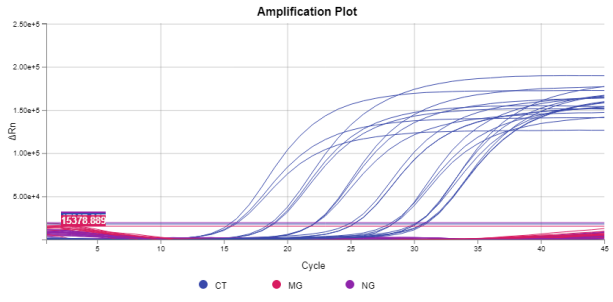
10 PERFORMANCE CHARACTERISTICS

10.1 Analytical sensitivity

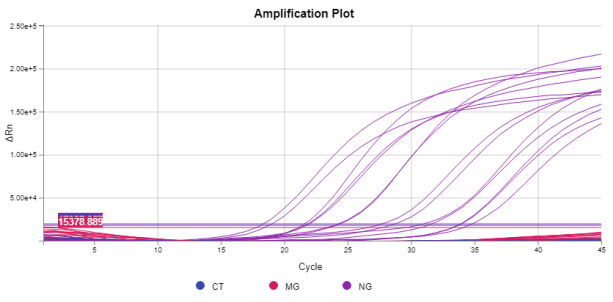
The analytical sensitivity of the **STI CNM Real Time PCR** kit was determined by performing three replicates of serial dilutions of synthetic fragments of each of the targets from 10^7 copies/rxn to 10^1 copies/rxn.

It has been established that the **STI CNM Real Time-PCR kit** has a limit of detection of 10 copies/reaction for *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Mycoplasma genitalium* (Figure 2).

A



B



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C

D

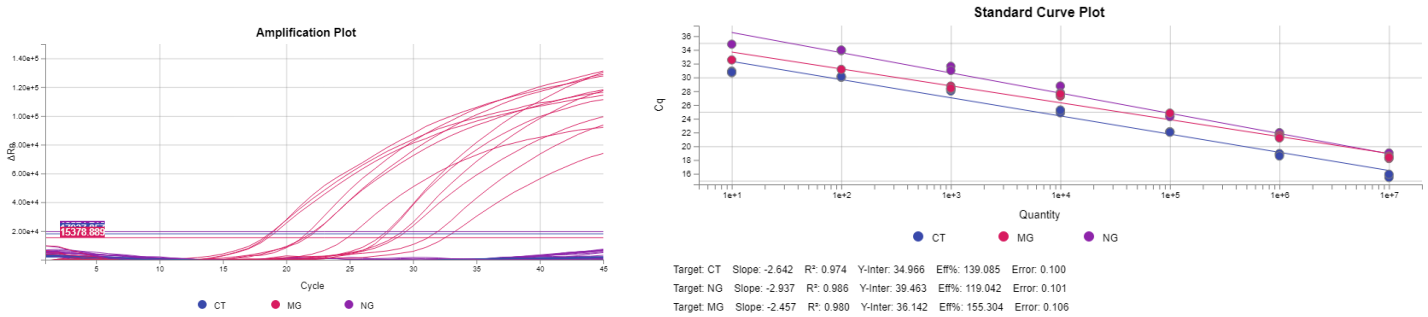

Figure 2: Serial dilutions from 10^7 copies/reaction up to 10^1 copies/reaction of synthetic fragments of *Chlamydia trachomatis* in the ROX (A) channel, *Neisseria gonorrhoeae* in the FAM (B) channel and *Mycoplasma genitalium* in the JOE (C) channel. Calibration lines obtained for the three targets (D). Experiment performed in Applied Biosystems QuantStudio™ 5 Real-Time PCR System.

The amplification efficiency for each of the targets was evaluated with three serial dilutions of a standard of STI from 10^7 copies/rxn to 10^1 copies/rxn. By adjusting the Cts data to a line, the amplification efficiency, R^2 and the slope were determined for each of the genes.

The *Chlamydia trachomatis* cryptic plasmid showed an efficiency of 139.08%, an R2 of 0.974 and a slope of -2.64. The *Neisseria gonorrhoeae* Opal gene showed an efficiency of 119.04%, an R2 of 0.986 and a slope of -2.93. The *Mycoplasma genitalium* MgPa gene showed an efficiency of 155.30%, an R2 of 0.98 and a slope of -2.457.

10.2 Analytical specificity

The specificity of the STI CNM test was confirmed by testing positive clinical samples for other pathogens related to sexually transmitted diseases that are included in the STD Direct DNA Flow Chip Kit. In addition, specificity tests were also performed against high-risk and low-risk genotypes of human papillomavirus and against other bacteria that share an ecological niche. The full list of organisms tested for cross-reactivity is shown in the following table.

Cross-reactivity test	
Microorganism	**Results**
HSV-1	Negative
HSV-2	Negative
T. pallidum	Negative
H. ducreyi	Negative
T. vaginalis	Negative
M. hominis	Negative
U. urealyticum	Negative
Candida albicans	Negative
Enterobacter cloacae	Negative
Escherichia coli	Negative
Klebsiella pneumoniae	Negative
Proteus mirabilis	Negative

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Staphylococcus aureus	Negative
Streptococcus pyogenes	Negative
Enterococcus faecium	Negative
Staphylococcus aureus	Negative
Enterococcus faecalis	Negative
Klebsiella oxytoca	Negative
Staphylococcus epidermis	Negative
Streptococcus penumoniae	Negative
Pseudomonas aeruginosa	Negative
HR genotype HR 16	Negative
HR genotype HR 18	Negative
HR genotype HR 26	Negative
HR genotype HR 31	Negative
HR genotype HR 35	Negative
HR genotype HR 35	Negative
HR genotype HR 51	Negative
HR genotype HR 52	Negative
HR genotype HR 53	Negative
HR genotype HR 56	Negative
HR genotype HR 59	Negative
HR genotype HR 68	Negative
HR genotype HR 73	Negative
HR genotype HR 82	Negative
LR genotype HR 6	Negative
LR genotype HR 11	Negative
LR genotype HR 42	Negative
LR genotype HR 43	Negative
LR genotype HR 54	Negative
LR genotype HR 61	Negative
LR genotype HR 62	Negative
LR genotype HR 81	Negative
LR genotype HR 67	Negative
LR genotype HR 70	Negative
LR genotype HR 71	Negative
LR genotype HR 72	Negative
LR genotype HR 84	Negative

No cross-reactions were detected with any of the following pathogens tested.

10.3 Repeatability

The repeatability was analyzed by testing the method 5 times for each of the targets included in the panel. For this purpose, a known concentration of synthetic DNA fragments mimicking each of the targets to be amplified was used. The test was performed by the same operator, in a single location and using the same reagent lot and the same platform. The platform used was Applied Biosystems QuantStudio™ 5 Real-Time PCR System and the results were analyzed with the version v. 2.4.3. The variability between tests was

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determined from the Cts values obtained from the repetitions. The coefficient of variation (CV) was calculated as standard deviation divided by the mean of the Cts, being 1.03% for *Chlamydia trachomatis*, 5.67% for *Neisseria gonorrhoeae* and 4.31% for *Mycoplasma genitalium*.

10.4 Reproducibility

The reproducibility of the method was analyzed by simulating the inter-laboratory variability, changing the operator, the equipment used in the process and the lots of PCR mix. Thirty-five purified DNA samples were tested using the Maxwell extraction system (Maxwell® 16 FFPE Tissue LEV DNA Purification Kit) of which 25 were positive for CT, NG or MG (presence of 4 coinfections) and 10 samples were negative.

Concordance was calculated with a kappa coefficient of 0.815, standard error of 0.1 and a 95% CI of 0.6141.016 showing a very good concordance strength for the **STI CNM Real Time PCR** kit.

	Laboratory 1		
Laboratory 2	positive	negative	Total
positive	**26**	**1**	27
negative	**2**	**10**	12
Total	28	11	39

Table 6. Reproducibility test for the targets included in the panel of STI CNM Real Time PCR.

10.5 Measurement range

The test's measurement interval was determined by using synthetic DNA fragments for each of the targets that are included in the STI CNM MMix.

STI CNM Real Time PCR kit has been shown to work correctly in the presence of synthetic fragments of each of the targets from 10^7 copies/reaction to 10 copies/reaction. (See section 10.1 Analytical sensitivity).

To determine the upper limit, serial dilutions from 10^9 copies/reaction to 10^8 copies/reaction of synthetic fragments of each target were tested, with 5 ng of human genomic DNA. Three replicas were tested at each level. The Cts values obtained are shown in the table below.

All PCRs were performed with the real time PCR system QuantStudio ™ 5 and were analyzed with the analysis and design software QuantStudio ™ 2.4.3

Template	Copies/test	Ct ROX (CT)	Ct FAM (NG)	Ct VIC (MG)
Chlamydia trachomatis	**10 (9)**	8.368906	Undefined	Undefined
		8.33922	Undefined	Undefined
		8.27948	Undefined	Undefined
	10 (8)	11.16678	Undefined	Undefined
		11.64812	Undefined	Undefined
		11.42841	Undefined	Undefined
Neisseria gonorrhoeae	**10 (9)**	Undefined	8.393139	Undefined
		Undefined	8.27919	Undefined
		Undefined	8.100005	Undefined
	10 (8)	Undefined	11.85441	Undefined
		Undefined	11.97664	Undefined
		Undefined	11.86866	Undefined
Mycoplasma genitalium	**10 (9)**	Undefined	Undefined	Undetermined
		Undefined	Undefined	8.69793
		Undefined	Undefined	Undetermined
	10 (8)	Undefined	Undefined	10.46096
		Undefined	Undefined	10.20658
		Undefined	Undefined	11.32994

It has been established that the STI CNM Real Time PCR kit has a measurement range of 10^9 copies/reaction to 10 copies/reaction for *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and 10^8 copies/reaction to 10 copies/reaction for *Mycoplasma genitalium* without producing cross-reactions.

10.6 Clinical sensitivity and specificity

STI CNM Real Time PCR kit was validated from DNA purified by any of the extraction methods aforementioned. The diagnostic capability of STI (CT-NG-MG) **Real Time PCR** kit was evaluated by studying its diagnostic sensitivity and specificity. These two parameters are defined and calculated as follows:

- The **diagnostic specificity** is expressed as a percentage (numerical fraction multiplied by 100), calculated as 100 x the number of true negative values (TN) divided by the sum of true negative values (TN) plus the number of false positive (FP) values, or 100 × TN/ (TN + FP).

- The **diagnostic sensitivity** is expressed as a percentage (numerical fraction multiplied by 100), calculated as 100 × the number of true positive values (TP) divided by the sum of true positive values (TP) plus the number of false negative values (FN), or 100 × TP/ (TP + FN).

A total of 163 clinical samples of different origin from different hospitals were analyzed in a retrospective study: Hospital de La Princesa (Madrid), Hospital Clínico San Cecilio (Granada), Hospital de Valme (Seville) and Hospital Universitario de Son Espases (Palma). Of these samples, 152 were positive and 11 were negative. The comparative study was performed using the STD Direct DNA Flow Chip Kit as a reference method (Vitro. S.A.) with CE-IVD marking.

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Organism	TN	FP	TP	FN	Diagnostic Specificity	95% CI	Diagnostic Sensitivity	95% CI
N. gonorrhoeae	117	0	45	1	100.00%	96.03-100%	97.82%	87.03-99.88%
M. genitalium	140	0	23	0	100.00%	96.67-100%	100%	82.19-100%
C. trachomatis	65	0	97	1	100.00%	93.04-100%	99%	93.63-100%

Table 7. Clinical specificity and sensitivity of STI CNM Real Time PCR kit.

Organism	TN	FP	TP	FN	PPV	95% CI	NPV	95% CI
N. gonorrhoeae	117	0	45	1	100.00%	90.2-100%	99.15%	94.67-100%
M. genitalium	140	0	23	0	100.00%	82.19-100%	100%	96.67-100%
C. trachomatis	65	0	97	1	100.00%	95.25-100%	98.5%	90.73-100%

Table 8. Positive predictive value and negative predictive value of STI CNM Real Time PCR kit.

Note: the results of the specifications (sensitivity and specificity) declared correspond to the total number of samples tested and the value may vary depending on the type of sample.

11 LIMITATIONS OF THE TEST

1. The results of the test must be evaluated by a healthcare professional in the context of medical history, clinical symptoms, and other diagnostic tests.

2. Use of inadequate samples: the method has been validated on the basis of purified genetic material from them. The clinical sample types that have been validated are: urine, semen, liquid-based cytologies; and urethral, endocervical, anal and pharyngeal swabs. The analysis of any other type of specimen not indicated can lead to wrong or inconclusive results due to PCR reaction inhibition by inhibiting chemical agents.

3. The correct performance of the test depends on the quality of the sample; the nucleic acid must be properly extracted from the clinical samples. Improper collection, storage and/or transport of samples can result in false negatives.

4. A low number of target copies below the detection limit can be detected, but the results may not be reproducible.

5. A positive test for STI CNM does not exclude the possibility that other pathogens are present in the clinical sample.

6. A negative result of the test does not exclude that there is an infection with STI CNM and it should not be used as the sole diagnostic method to establish a treatment or patient management regime.

7. A negative result of the test must be analyzed in the context of medical history of the patient and epidemiology.

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13 LABEL AND BOX SYMBOLS

IVD	In vitro diagnostic medical device		Expiration date
REF	Catalog number		Temperature limit
LOT	Lot code		Manufacturer
	Refer to the instructions for use		Sufficient content for <n> assays
	Material safety data sheet		Keep away from sunlight

14 CHANGELOG

Date	Description
2022/04/18	• Creation of the document.

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