*ampli*Cube STD Panel 1

MIKROGEN DIAGNOSTIK

IVD

Instructions for use (English)

1 Purpose

The *ampli*Cube STD Panel 1 is a qualitative in-vitro test for specific detection of the DNA of *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Mycoplasma genitalium* in urine samples (preferably first stream urine) or urogenital smears of human origin.

2 Field of Application

Chlamydia trachomatis and *Neisseria gonorrhoeae* are the most common sexually transmitted bacterial pathogens.
Chlamydia trachomatis is an obligate intracellular bacterium. In most cases the infection is without symptoms. In women an untreated infection can lead to *pelvic inflammatory disease* (PID) which can be associated with chronic symptoms and consequences such as ectopic and abdominal pregnancies and infertility. An infection with *Chlamydia trachomatis* can lead to neonatal conjunctivitis in newborns. In men the infection can manifest with the clinical symptoms of urethritis or epididymitis.
Neisseria gonorrhoeae is a Gram-negative diploid bacterium. The bacterium is the pathogen that causes gonorrhoea which in men can lead to epididymitis and in women to *pelvic inflammatory disease*, infertility and ectopic and abdominal pregnancies. Outside the genital area, *Neisseria gonorrhoeae* can cause infections around the anorectum, oropharynx and the eyes.
An infection with *Mycoplasma genitalium* in women can cause inflammations of the cervix, endometriosis, urethritis and pelvic inflammatory disease. In men the bacterium can lead to symptoms of urethritis. *Mycoplasma genitalium* is also linked to infections of the mucosal membranes in the respiratory and gastrointestinal tracts.

3 Test Principle

The test is a real-time PCR system. It uses specific primers and marked probes for amplifying and detecting DNA from *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Mycoplasma genitalium*. To ensure that the nucleic acids isolated from the patient sample do not contain any substances that inhibit PCR, an internal control (IC) is added to the sample during DNA isolation. This IC is amplified and detected in the same PCR batch. This enables false negative test results due to inhibition of the PCR reaction to be ruled out. At the same time, the IC serves as evidence of nucleic acid extraction from the patient sample.
Probes for detecting pathogen-specific DNA are marked with the reporter dyes FAM (*Chlamydia trachomatis*), HEX (*Neisseria gonorrhoeae*) and ATTO Rho12 (*Mycoplasma genitalium*) and the probes for detecting the internal control are marked with ATTO 647N. This allows simultaneous detection of all target sequences in a single reaction mixture.
The Ct value (*cycle threshold*) describes the part of the curve in which the fluorescence rises exponentially above the background value for the first time.

4 Reagents

4.1 Package contents

The reagents of one pack are sufficient for 50 assays.
Each set of reagents contains:

P&P MIX	**150 µl** primer & probe mix for STD Panel 1 and internal control (**green lid**)
ENZYME	**600 µl** enzyme mix (**white lid**) Contains DNA polymerase. (**component is stained blue**)
CONTROL INT	**250 µl** internal control (**colourless lid**)
CONTROL +	**170 µl** positive control (**red lid**)
CONTROL -	**2 × 1800 µl** negative control (**blue lid**)
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4.2 Additionally required reagents, materials and equipment

- MIKROGEN *ampli*Cube Color Compensation for Light Cycler® 480 II (Roche)
- Commercial nucleic acid isolation kit. The following nucleic acid extraction system is recommended:
  MagNAPure® Compact, Total Nucleic Acid Kit I (Roche)
- Real-Time Cycler. The following cycler is recommended:
  Light Cycler® 480 II (Roche)
- 96 well PCR plates and films or reaction tubes (PCR-clean), dependent on the cycler
- Micropipettes with single-use tips with filter 10 µl, 20 µl, 100 µl and 1000 µl
- Vortex mixer
- Mini centrifuge
- Possibly plate centrifuge
- Single-use gloves, powder-free
- Cooling block

5 Shelf life and Handling

- Store reagents between -25°C and -18°C before and after use.
- Repeated thawing and freezing of the components (more than ten times) must be avoided. It is recommended to aliquot the test components after the first thawing.
- Always appropriately chill reagents during the working steps (+2°C – +8°C).
- Keep the kit components away from direct sunlight throughout the test procedure.
- Before starting the test, all reagents must be completely thawed, mixed (briefly vortex) and centrifuged.
- The packages have an expiry date, after which no further guarantee of quality can be given.
- The test must only be performed by trained, authorised and qualified personnel.
- Substantial changes made by the user to the product or the directions for use may compromise the intended purpose of the test specified by Mikrogen.
- Cross-contamination can lead to false test results. Add patient samples and controls carefully. Ensure that the reaction mixtures are not transferred to other wells.

6 Warnings and Safety Precautions

- Only use for in vitro diagnostics.
- All patient samples must be treated as potentially infectious.
- Suitable single-use gloves must be worn throughout the entire test procedure.
- All reagents and materials coming into contact with potentially infectious samples must be treated with suitable disinfectants or be disposed of according to laboratory guidelines. The concentration specifications and incubation times of the manufacturer must be taken into consideration.
- Do not replace or mix the reagents with reagents from other kit batches, other MIKROGEN PCR kits or with reagents from other manufacturers.
- Read through and carefully follow all instructions before performing the test. Deviations from the test protocol described in the instructions for use can lead to false results.

7 Sampling and Reagent Preparation

7.1 Sample material

The starting material for the *ampli*Cube STD Panel 1 is DNA extracted from urine samples (preferably first stream urine) or urogenital smears of human origin. The quality of the nucleic acid preparation affects the test result. It must be ensured that the extraction method selected is compatible with the real-time PCR technology.

7.2 Extraction of nucleic acids

Extract the nucleic acids from the patient sample and the negative control (NC). We recommend a starting volume for the extraction of 200 µl and an elution volume of 50 µl. Follow the instructions from the manufacturer of the extraction kit.

1. Thaw the internal control (IC) (colourless lid) and the negative control (NC) (blue lid).
   Ensure that the IC and the NC are completely thawed. Mix the IC and the NC before use by briefly vortexing and then briefly centrifuge.

2. For the extraction, add 5 µl IC to each patient sample and the NC. The IC should be added to the sample/lysis buffer mix and not directly to the sample material. (Note: the IC cannot be used in the PCR without extraction!)
3. Extract the patient sample and the NC. (Note: the NC cannot be used in the PCR without extraction!)
4. The positive control is not extracted.

The following nucleic acid extraction system is recommended and was used for the performance assessment:

Extraction system	Sample volume	Elution volume
MagNAPure Compact (Roche) Total Nucleic Acid Kit I	200 µl	50 µl

If you would prefer to use other extraction methods, please contact the manufacturer to clarify the compatibility.

7.3 Preparing the master mix

1. Thaw the primer & probe mix (green lid) and the enzyme mix (white lid). Protect the reagents from light while doing so. **Ensure that the reagents are completely thawed. Mix the reagents before use by briefly vortexing and then briefly centrifuge.**
2. Prepare the master mix using the following pipetting scheme:

Components	Master mix for 1 reaction
Primer & probe mix	3 µl
Enzyme mix	12 µl
Total volume	15 µl

3. Mix the entire master mix by vortexing and then briefly centrifuge.
4. Introduce 15 µl master mix for each PCR reaction.

7.4 Preparing the PCR reaction

1. Thaw the positive control (PC) (red lid). **Ensure that the reagents are completely thawed. Mix the reagents before use by briefly vortexing and then briefly centrifuge.**

Components	1 reaction
Master mix from 7.3	15 µl
Sample eluate or eluate of the NC or the PC	10 µl

2. Pipette 10 µl of the sample eluate into the master mix.
3. Pipette 10 µl of the positive control (not prepared) into the master mix.
4. Pipette 10 µl of the eluate of the negative control into the master mix.

Every run must include a positive and a negative control.
Seal the PCR plate with an adhesive optically clear film or the reaction tube with the lid provided.

⚠ PCR plates and tubes must be vortexed for <u>at least 10 sec</u> at maximum speed, followed by brief centrifugation.

8 Programming the real-time cycler

The *ampli*Cube STD Panel 1 was evaluated with the LightCycler® 480 Instrument II (Roche).

8.1 Setting the detection channels

	Chlamydia trachomatis	*Neisseria gonorr.*	*Mycoplasma genitalium*	Internal control (IC)
Reporter dye	FAM	HEX	ATTO Rho12	ATTO 647N
Colour	Green	Yellow	Orange	Red
Emission	510 nm	580 nm	610 nm	660 nm
Quencher	[none]	[none]	[none]	[none]

Details of the wavelengths for the detection channels refer to the LightCycler® 480 II.
For the LightCycler® 480 II, it is necessary to first use colour compensation, which is provided by Mikrogen.

8.2 PCR program

Reverse transcription	50°C	8 minutes
Denaturation	95°C	3 minutes
Amplification	**45 cycles**	
• Denaturation	95°C	10 seconds
• Annealing/elongation	60°C	45 seconds

Essential information about programming the different real-time cyclers can be found in the instructions for the cycler used. For specific information about programming the real-time PCR cycler when using the *ampli*Cube STD Panel 1, please contact the manufacturer.

9 Results

The data analysis for the LightCycler® 480 II uses the *Abs Quant/2nd Derivative Max* method.

9.1 Validation

1. The negative control must be below the *threshold*. If this control is contaminated (positive curve), the test run cannot be evaluated.
2. The positive control must have a positive curve. The Ct value for the positive control must be < 33. A positive control outside this range indicates there is a problem with the amplification.
3. The internal control must have a positive curve for negative samples and the negative control. A deviation in the curve progression of the IC in negative samples in comparison with the negative control indicates that there is a problem in the extraction of the nucleic acids or inhibition of the PCR.

9.2 Evaluation

Signals above the *threshold* are evaluated as positive results. Empty fields in the table are considered a negative result.

	Chlamydia trachomatis	*Neisseria gonorr.*	*Mycoplasma genitalium*	Internal control (IC)
Colour				
Green	Positive			
Yellow		Positive		
Orange			Positive	
Red				Positive*

*In the case of positive signals in the detection channels for the pathogens, the signal for the internal control is not required for the test interpretation. A high pathogen load in the patient sample can lead to a reduced or missing signal for the internal control.

10 Limitations and Restrictions of the Method

- Test results must always be viewed in the context of the clinical situation. Therapeutic consequences of the findings must be linked to the clinical data.
- A negative *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and/or *Mycoplasma genitalium* test result cannot rule out an infection with the particular pathogen.

11 Performance Characteristics

11.1 Diagnostic sensitivity and specificity

The sensitivity and specificity were determined using defined positive and defined negative samples.

Table 1: Defined positive samples

*ampli*Cube STD Panel 1	*Chlamydia trachomatis* (n=10)	*Neisseria gonorrhoeae* (n=11)	*Mycoplasma genitalium* (n=10)
Negative	0	0	0
Positive	10	11	10
Sensitivity	**100%**	**100%**	**100%**

Table 2: Defined negative samples

*ampli*Cube STD Panel 1	*Chlamydia trachomatis* (n=50)	*Neisseria gonorrhoeae* (n=49)	*Mycoplasma genitalium* (n=50)
Negative	50	49	50
Positive	0	0	0
Specificity	**100%**	**100%**	**100%**

11.2 Analytical sensitivity

The limit of detection (LoD) of the *ampli*Cube STD Panel 1 was determined using a dilution series of plasmid DNA of known concentration on a LightCycler® 480 II System (Roche). The 95% limit of detection was determined using probit analysis with CombiStats™ Version 5.0 software (Council of Europe).

Table 3: Limit of detection (LoD)

	Chlamydia trachomatis	*Neisseria gonorrhoeae*	*Mycoplasma genitalium*
LoD 95% limit of detection Genome/PCR	16.00 (9.41 – 39.39)	11.29 (7.09 – 25.70)	11.97 (7.10 – 28.31)

11.3 Analytical specificity

The BLAST trial (www.ncbi.nlm.nih.gov/blast/) shows that the selected primers and probes of the *ampli*Cube STD Panel 1 specifically detect the selected pathogens.
Furthermore, the specificity was determined by studying genomic DNA/RNA of other human pathogenic bacteria and viruses.

Table 4: Bacteria and viruses that were tested to demonstrate the analytical specificity of the *ampli*Cube STD Panel 1.

Bacteria
Aeromonas hydrophilia
Bordetella pertussis
Camplyobacter jejuni
Campylobacter coli
Citrobacter freundii
Clostridium difficile
Clostridium perfringens
EHEC stx+
EIEC
Enterococcus faecalis
ETEC
Haemophilus influenzae
Klebsiella oxytoca
Klebsiella pneumoniae
Legionella pneumophila
Moraxella catarrhalis
Morganella morganii
Mycoplasma pneumoniae
Neisseria cinerea
Proteus mirabilis
Proteus vulgaris
Salmonella typhimurium

Viruses
Adenovirus A
Adenovirus Serotype 1 (C)
Adenovirus Serotype 3 (B)
Astrovirus
Coronavirus 229E
Coronavirus NL63
Coronavirus OC43
Cytomegalovirus
Enterovirus 68
Epstein-Barr Virus
Herpes simplex virus 1
Herpes simplex virus 2
Human Metapneumovirus A
Influenza A virus
Influenza B virus
Measles virus
Mumps virus
Norovirus G1
Norovirus G2
Parainfluenza 1
Parvovirus B19
RSV A
RSV B
Rotavirus
Varicella-zoster virus

None of these samples generated a positive signal. The primers and probes used in the *ampli*Cube STD Panel 1 showed no cross-reaction with the pathogens listed in Table 4. The internal control (IC) was valid in all tests.

12 Literature

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We will be pleased to send you additional literature on request.

13 Explanation of symbols

Σ	Content is sufficient for <n> formulations Number of formulations
P&P MIX	Primer & Probe mix
ENZYME	Enzyme mix
CONTROL INT	Internal control
CONTROL +	Positive control
CONTROL -	Negative control
INSTRU	Instructions for use
	Follow the instructions for use
CONT	Contents, contains
IVD	In vitro diagnostic agent
LOT	Batch/version number
REF	Order number
	Use by Expiry date
y°C x°C	Store between x°C and y°C
	Manufacturer

14 Manufacturer and version data

***ampli*Cube STD Panel 1**	Article no. **50301**
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