1 LIST OF PRODUCT VARIANTS

Product name	Packaging volume	REF
eDetect CT-NG QL PCR Kit	25 reactions	EDA1000

2 INTENDED PURPOSE AND USE

The intended use of a device is determined by the medical context and clinical circumstances in which the device is utilized.

Target of detection	*Chlamydia trachomatis* (including Swedish New and Finnish Variant) and *Neisseria gonorrhoeae*
Automatic/manual detection	Manual
Type of analysis	Qualitative
Validated sample types	DNA extracted from urogenital swabs (urethral, vaginal) and urine (urine sediment)
Specific information	Examining individuals exhibiting symptoms of a urogenital tract infection, such as vaginal/urethral discharge, discomfort or burning sensation during urination, ulcers or rash, pelvic pain in women, testicular pain or swelling in men; partners of individuals diagnosed with *Chlamydia trachomatis* or *Neisseria gonorrhoeae* infection; screening tests for individuals based on the healthcare guidelines of their respective countries.
Indications	*In vitro* diagnostic medical device
Regulatory status	Regulation (EU) 2017/746 (certification in process)
Functions	Diagnosis and help with the diagnosis of infection
Intended user	For professional use in laboratories with trained personnel
The principle of the test	Real-time polymerase chain reaction (PCR) - amplification of specific target sequence and detection using TaqMan fluorophore-based detection probes

3 TECHNICAL SPECIFICATION

Target sequence	16S rRNA gene and cryptic plasmid sequence for *Chlamydia trachomatis* 16S rRNA gene and ORF1 gene for *Neisseria gonorrhoeae*			
Analytical specificity	*Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG)			
Analytical sensitivity: Limit of Detection (LoD with 95% probability)	**Sample processing**	**Target**	**Sensitivity**	**Performed on**
	Without extraction*	CT	0,2 ± 0,03 cp/µl	Amplirun® Chlamydia trachomatis DNA control, Vircell
		NG	0,2 ± 0,01 cp/µl	Amplirun® Neisseria gonorrhoeae DNA control, Vircell
	** PCR performed directly on DNA control*			
Measuring range	LoD to 10^6 cp/µl			
Precision: Repeatibility and reproducibility	Maximal variability within Ct +/- 1,7 Ct trough three tested batches			
Diagnostic sensitivity	*100* % - *CT*, 100 % - *NG*			
Diagnostic specificity	*100* % - *CT*, 98 % - *NG*			
Positive predictive value	*100* % - *CT*, 95 % - *NG*			
Negative predictive value	*100* % - *CT*, 100 % - *NG*			
Metrological traceability	Not applied - qualitative detection only			
Extraction/Inhibition Control	Internal exogenous control (EXO-IC): control of DNA extraction efficiency, control of PCR inhibition Internal endogenous control (ENDO-IC): monitor the quality and adequacy of sample collection, control of cellular material presence in the sample (sampling quality)			
Validated specimen/transport media	ESwab® Liquid Based Collection and Transport, COPAN			
Validated extraction methods	NucleoSpin®Tissue (240), Machery-Nagel			
Applicable instruments, detection channels	**Instrument name**	**CT**	**NG**	**Internal Exogenous Control (EXO-IC)** / **Internal Endogenous Control (ENDO-IC)**
	Bio Rad CFX Opus 96 Real-Time PCR System	FAM	ROX	HEX / Cy5
	The kit has been validated on the devices listed in the table above. However, the kit can also be used on other PCR machines, the exact settings, validation of the protocols and safety use are the responsibility of the user.			

4 ANALYTICAL INTERFERENCE

Specimen/media tested In urine	Monitored interference	Specimen/media tested In eSwab medium	Monitored interference
2 % hemoglobin	none	1 % hemoglobin	none
10 µg/ml ibuprofen	none	0,1 % mucin	none
0,01 % glucose	none	0,25 % water-based lubricant	none
0,001 % glucose	none	0,25 % oil-based cream	none
Vitamins and minerals*	none		
0,2 % albumin	none		

** The Supradyn CoQ energy complex dissolved in the urine to evaluate potential interference of vitamins A, B1, B2, B3/PP, B5, B6, B9, B12, C, D3, E, K1, biotin, coenzyme Q10 minerals Ca, Cu, I, Fe, Mg, Mn, Mo, Se, Zn.*

5 PACKAGING CONTENTS

Components	Table of Contents	Colour of the lid	Guaranteed volume	Number of tubes
Polymerase	Lyophylized, containing UDG	transparent	25 rx	1
PreMix CT-NG	Mixture of target-specific primers and probes in buffer	transparent	540 µl	1
PC CT-NG	DNA oligomeres in stabilisation buffer	transparent	100 µl	1
NC	Nuclease free H_2O (for negative control of amplification)	transparent	100 µl	1
EXO-IC	Plasmid DNA in stabilisation buffer	transparent	500 µl	1

NOTE: The packaging contents also:

- *"MasterMix" label to relabel the Polymerase tube after dissolving the Polymerase in the PreMix*
- *Desiccant*

Description of reagents and associated limitations

The mixtures in this product are not classified as hazardous according to Regulation (EC) No 1272/2008.

6 INFORMATION ON CALIBRATORS

No calibrators - only qualitative detection.

7 STORAGE AND TRANSPORT CONDITIONS

Storage conditions	-20 ± 5 °C
Transport conditions	Room temperature for max 10 days
Stability during use	3 thaws of a particular tube, 1 hour at room temperature

NOTE: Selected components of the kit (PreMix) are to be kept away from light.

8 WORKFLOW

Collection, transport, handling of samples

- Samples intended for NA extraction must adhere to professional regulations during collection, transportation and storage.
- Samples designated for NA extraction should be transported to the laboratory and processed promptly upon arrival.

COMMENT: For more information, please refer to the Instructions for Use of the respective extraction kit.

Purification of nucleic acids

1. Prepare the samples according to the Instructions for Use of the extraction kit.
   COMMENT: To maximize sensitivity, prefer the largest possible input extraction volume within the volume range recommended by the extraction kit manufacturer.
2. Thaw the EXO-IC, vortex gently and centrifuge briefly.
3. At the beginning of the extraction process, add the EXO-IC directly to the sample so that 1 µl of the resulting elution volume contains 0.1 µl of EXO-IC:

Elution volume	25 µl	50 µl	100 µl
EXO IC	2,5 µl	5 µl	10 µl

4. Proceed with the extraction according to the appropriate protocol.

Procedure for preparing the PCR

1. Thaw the reagents completely.
   COMMENTS:
   - *Thaw the reagents gradually (in a refrigerator at 2 - 8 °C or on ice) to prevent sudden temperature changes that could damage the components (e.g. enzyme).*
   - *When handling the PreMix, limit its exposure to light to avoid reducing the intensity of the fluorescence signal.*
2. Briefly centrifuge Polymerase (max. 5 s).
3. Vortex gently and centrifuge briefly PreMix and add 540 µl of PreMix into Polymerase tube.
4. Leave to dissolve for 1 min at room temperature, then vortex gently and centrifuge briefly. Relabel the Polymerase tube with MasterMix label with indicated LOT and date of resolving on the label.
5. Add 20 µl of MasterMix to the PCR tubes.
   COMMENT: Store unused MasterMix at -20 ± 5 °C.
6. Add 5 µl of extracted nucleic acid/Positive Control/Negative Control to each PCR tube and mix by pipetting. The total volume of the reaction mixture is 25 µl.
7. Cap the tubes, centrifuge briefly, place them in a real-time PCR machine and amplify according to the following PCR profile.
   COMMENT: It is recommended to include at least 1 negative control and at least 1 positive control for each PCR run. For more information, see Chapter 10 Validity of PCR run.

Amplification profile

Follow the manufacturer's guidelines when configuring the instrument for analysis.

Universal PCR Profile

Step	Process	Temperature [°C]	Time	Cycles	Increase in fluorescence
1	UDG decontamination	37	120 sec		
2	Initial denaturation	95	120 sec		
3	Denaturation	95	10 s	5	
	Annealing	62	40 s		
4	Denaturation	95	5 s	35	
	Annealing	60	40 s		FAM, HEX, ROX, Cy5

9 INTERPRETATION OF RESULTS

Channel FAM (CT)	Channel ROX (NG)	Channel HEX (EXO IC)	Channel Cy5 (ENDO IC)	Sample results	Interpretation
+	+	+/-[1]	+/-[1]	Valid	*Chlamydia trachomatis* and *Neisseria gonorrhoeae* positive
+	-	+/-[1]	+/-[1]	Valid	*Chlamydia trachomatis* positive
-	+	+/-[1]	+/-[1]	Valid	*Neisseria gonorrhoeae* positive
-	-	+	+	Valid	*Chlamydia trachomatis* and *Neisseria gonorrhoeae* negative
-	-	-	+	Unvalid	Low extraction efficiency or inhibition of the RT-PCR reaction. Repeat nucleic acid extraction.
-	-	+	-	Unvalid	Absence of human DNA in primary sample caused by improper collection. Repeat the sampling and extraction procedure.[2]
-	-	-	-	Unvalid	Repeat the procedure

[1]For positive samples, amplification of the internal controls may be negatively affected by amplification of pathogen targets. Positive samples are considered positive even if the EXO-IC and ENDO-IC extraction control fails.
[2]Keep in mind, that in case of extraction from non-cellular primary samples (e.g. urine), the Cy5 signal can be negative due to the low amount of human DNA in the sample. However, the result is still valid.

10 VALIDITY OF PCR RUN

Overall detection validity

	Signal	Channel	Validity of run	Recommendation
Positive Control	+	FAM, ROX	Valid	-
Positive Control	-		Unvalid	Repeat PCR
Negative Control	-		Valid	-
Negative Control	+		Unvalid	Repeat PCR

11 REQUIRED MATERIAL AND EQUIPMENT NOT INCLUDED IN THE PACKAGE

Consumables

96-well PCR plates or PCR strips or tubes compatible with the device used, pipetting tips with filter, powder-free gloves, biohazard bin, nuclease-free water.

Equipment

Real-time PCR instrument (see Chapter 3 Technical Specification), nucleic acid extraction system or kit (see Chapter 3 Technical Specification), benchtop centrifuge (for 96-well PCR plates or 0,2 ml strips or tubes), vortex, freezer (-20 ± 5 °C), refrigerator (5 ± 3 °C), automatic pipettes, racks.

12 WARNINGS and PRECAUTIONS

- Use disposable protective gloves and laboratory clothes and protect your eyes while handling samples and reagents. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid samples and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- Safety Data Sheets (SDS) are available on request.
- Use sterile pipette tips with aerosol barriers and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components gradually at 2-8 °C or on ice before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- The workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where the previous step was performed.
- Any serious adverse event that occurred in connection with the product must be reported to the manufacturer and to the competent authority of the member state where the user or patient is established.

13 PROCEDURE LIMITATIONS

- Do not use a kit after its expiration date.
- Do not use kit components that are damaged upon receipt.
- Do not mix components from different kit lots.
- Use of this product should be limited to personnel trained in NA amplification techniques.
- The kit should be used in accordance with regulatory requirements and manufacturer's instructions. Do not use the kit for purposes beyond its intended use or in settings where regulatory compliance cannot be assured.
- The results generated by the multiplex PCR assay should be interpreted in conjunction with other clinical and laboratory findings. Positive or negative result from the assay should not be considered solitary but should be integrated into the overall diagnostic or clinical assessment. Negative result does not rule out infection with a given pathogen.
- Variations in sample type and quality, storage conditions, or environmental factors (e.g., temperature, humidity) may affect the performance of the assay. Please follow the instruction above.
- Mutations in highly conserved regions of the bacterial genome, which are targeted by the primers and/or probes of the eDetect CT-NG QL PCR Kit, may rarely occur, potentially resulting in failure of pathogen detection.

14 DISPOSAL

Dispose of all specimens and unused reagents in accordance with local regulations.

15 EXPLANATION OF SYMBOLS

Symbol	Explanation	Symbol	Explanation
CE	This product is in compliance with relevant EU requirements	LOT	Batch number
IVD	*In vitro* diagnostic medical device	Σ	Content sufficient for n-tests
REF	Catalogue number		Temperature limitation
	Manufacturer		Expiry date
	Read the electronic Instructions for Use	UDI	Unique Device Identifier (UDI)

16 REFERENCES

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17 CHANGES MADE IN THE LATEST VERSION

- This is the first version of the document

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