# eDetect MG-MH-Uspp QL PCR Kit

DEMO

## 1 LIST OF PRODUCT VARIANTS

| Product name | Packaging volume | REF |
|---|---|---|
| eDetect MG-MH-Uspp QL PCR Kit | 25 reactions | EDA1050 |

## 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** | *Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma spp.* |
| **Automatic/manual detection** | Manual |
| **Type of analysis** | Qualitative |
| **Validated sample types** | DNA extracted from urogenital swabs (urethral, vaginal), rectal swab, sperm, nasopharyngeal (oral) swab, eye swab, LBC, and urine (urine sediment) |
| **Specific information** | Examining individuals exhibiting symptoms of a urogenital tract infection.<br>Mycoplasma genitalium: urethritis in men (dysuria, penile discharge); cervicitis, pelvic inflammatory disease (PID), and infertility in women.<br>Mycoplasma hominis: bacterial vaginosis, postpartum infections, complications in immunocompromised individuals, pelvic inflammatory disease and infertility.<br>Ureaplasma spp.: urethritis, bacterial vaginosis, adverse pregnancy outcomes, neonatal infections.<br>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

<table>
<tr><td colspan="2">Target sequence</td><td colspan="5">MG-219 gene for Mycoplasma genitalium<br>YidC gene for Mycoplasma hominis<br>UreD gene for Ureaplasma spp</td></tr>
<tr><td rowspan="5">Scientific validity data</td><td>Specificity</td><td colspan="5">Mycoplasma genitalium (MG), Mycoplasma hominis (MH), Ureaplasma spp (Uspp), 100%</td></tr>
<tr><td rowspan="4">Sensitivity (LoD with 95% probability)</td><td>Sample processing</td><td>Target</td><td>Sensitivity</td><td colspan="2">Performed on</td></tr>
<tr><td rowspan="3">Without extraction*</td><td>MG</td><td>1,210 cp/µl</td><td colspan="2">Amplirun® Mycoplasma genitalium DNA control, Vircell</td></tr>
<tr><td>MH</td><td>1,420 cp/µl</td><td colspan="2">Amplirun® Mycoplasma hominis DNA control, Vircell</td></tr>
<tr><td>Uspp</td><td>0,760 cp/µl</td><td colspan="2">Amplirun® Ureaplasma urealyticum DNA control, Vircel<br>Amplirun® Ureaplasma parvum DNA control, Vircel</td></tr>
<tr><td colspan="6">* PCR performed directly on DNA control<br>COMMENT: The data based on test results during development. The data will be updated based on the testing of the first produced batches.</td></tr>
<tr><td colspan="2">Analytical specificity</td><td colspan="5" rowspan="2">Not determined yet.</td></tr>
<tr><td colspan="2">Analytical sensitivity</td></tr>
<tr><td colspan="2">Diagnostic specificity</td><td colspan="5" rowspan="4">Not determined yet.</td></tr>
<tr><td colspan="2">Diagnostic sensitivity</td></tr>
<tr><td colspan="2">Positive predictive value</td></tr>
<tr><td colspan="2">Negative predictive value</td></tr>
<tr><td colspan="2">Metrological traceability</td><td colspan="5">Not applied - qualitative detection only</td></tr>
<tr><td colspan="2">Extraction/Inhibition Control</td><td colspan="5">Internal exogenous control (EXO-IC): control of DNA extraction efficiency, control of PCR inhibition<br>Internal endogenous control (ENDO-IC): monitor the quality and adequacy of sample collection, control of cellular material presence in the sample (sampling quality)</td></tr>
<tr><td colspan="2">Validated extraction methods</td><td colspan="5">NucleoSpin®Tissue Genomic DNA Extraction kit</td></tr>
<tr><td colspan="2" rowspan="3">Applicable instruments</td><td>Instrument name</td><td>MG</td><td>MH</td><td>Uspp</td><td>Internal Exogenous Control (EXO-IC)</td><td>Internal Endogenous Control (ENDO-IC)</td></tr>
<tr><td>Bio Rad CFX Opus 96 Real-Time PCR System</td><td>FAM</td><td>Cy5.5</td><td>ROX</td><td>HEX</td><td>Cy5</td></tr>
<tr><td colspan="6">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.</td></tr>
</table>

## 4 INTERFERENCE

Not determined yet.

## 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 MG-MH-Uspp** | Mixture of target-specific primers and probes in buffer | transparent | 540 µl | 1 |
| **PC MG-MH-Uspp** | DNA oligomeres in stabilisation buffer | transparent | 100 µl | 1 |
| **NCE** | Nuclease free $H_2O$, for negative control of extraction (in volume for 10 extractions with entering volume 200 µl) | transparent | 1000 µl | 1 |
| **NCA** | 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*
- *Dessicant*

### 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 controls 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, Cy5.5, ROX, HEX, Cy5 |

## 9 INTERPRETATION OF RESULTS

| Channel | | | | | Sample results | Interpretation |
|---|---|---|---|---|---|---|
| FAM (MG) | Cy5.5 (MH) | ROX (Uspp) | HEX (EXO-IC) | Cy5 (ENDO-IC) | | |
| + | + | + | +/-[1] | +/-[1] | Valid | MG, MH and Uspp positive |
| + | + | - | +/-[1] | +/-[1] | Valid | MG and MH positive |
| + | - | + | +/-[1] | +/-[1] | Valid | MG and Uspp positive |
| - | + | + | +/-[1] | +/-[1] | Valid | MH and Uspp positive |
| + | - | - | +/-[1] | +/-[1] | Valid | MG positive |
| - | + | - | +/-[1] | +/-[1] | Valid | MH positive |
| - | - | + | +/-[1] | +/-[1] | Valid | Uspp positive |
| - | - | - | + | + | Valid | MG, MH, Uspp 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, Cy5.5 | 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 or 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 pathogen genome, which are targeted by the primers and/or probes of the eDetect MG-MH-Uspp 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


- European Commission. (2017). Regulation (EU) 2017/746 of the European Parliament and of the Council of 5 April 2017 on in vitro diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU. Official Journal of the European Union, L 117/1.
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- World Health Organization. (2021). *Guidelines for the management of symptomatic sexually transmitted infections*. Geneve: WHO.
- Jensen J. S. et al. *European guideline on the management of Mycoplasma genitalium infections*, European Academy of Dermatology and Venereology, 2022, 36, 641–650
- Combaz N., Kuhn A. *A Systematic Review of Mycoplasma and Ureaplasma in Urogynaecology*. Geburtsh Frauenheilk 2017; 77: 1299-1303
- Beeton M. L. et al. *The Role of Ureaplasma spp. in the Development of Nongonococcal Urethritis and Infertility among Men*. Clinical Microbiology Reviews 2019, 32, 4
- World Health Organization. (2024). *Recommendations for the treatment of Trichomonas vaginalis, Mycoplasma genitalium, Candida albicans, bacterial vaginosis and human papillomavirus (anogenital warts).* Geneve: WHO.


## 17 CHANGES MADE IN THE LATEST VERSION

- This is the first version of the document


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