RevoDx CT/NG plus Mycoplasma Hominis qPCR Kit

Instruction for Use

Qualitative Detection of Chlamydia Trachomatis, Neisseria Gonorrhoeae and Mycoplasma Hominis DNA

For in vitro diagnostic use

For professional use only

Product numbers:
IP202415-50 – 50 tests
IP202415-100 – 100 tests
IP202415-500 – 500 tests

RUO

Product Components

	Component Name	50 Tests	100 Tests	500 Tests
1	STI-31 RM 1	700 µl	1400 µl	5 x 1400 µl
2	STI-31 RM 2	50 µl	100 µl	500 µl
3	STI-31 Positive Control	100 µl	100 µl	200 µl
4	STI-31 Negative Control	100 µl	100 µl	200 µl

Transport, Storage and Stability

The kits may be shipped at +2°C to +8°C. All components of RevoDx CT/NG plus Mycoplasma Hominis qPCR Kit should be stored at -25°C to -15°C. Storage at higher temperatures should be avoided. If properly stored, all kit components are stable until the expiration date printed on the product label. STI-31 RM 1 vials should not be freeze-thawed more than 3 times; as this may reduce the sensitivity. Otherwise, divide them into conveniently sized aliquots, and store at -25°C to -15°C.

Intended Use

RevoDx CT/NG plus Mycoplasma Hominis qPCR Kit is a real-time PCR test intended for the qualitative detection and identification of nucleic acids of the Chlamydia Trachomatis, Neisseria Gonorrhoeae and Mycoplasma Hominis in urine samples or urogenital swabs or cervical swabs from both symptomatic and asymptomatic patients.

Positive results do not rule out co-infection with other pathogens. The agent detected may not be the definite cause of disease. Negative results do not preclude the infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

RevoDx CT/NG plus Mycoplasma Hominis qPCR Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

Product Use Restrictions

- For prescription use only
- For research use only
- Potential mutations in the target regions of the pathogen genomes covered by the oligos in the kit may lead to false negative test results.
- This kit has been validated for use with urine samples or urogenital swabs or cervical swabs. Test with other sample types may result in inaccurate results.
- PCR inhibitors in eluates may lead to false negative or invalid test results.
- Reliable results depend on proper specimen collection, transport, storage and handling methods.
- It is intended for professional use by properly trained personnel.
- Follow the instructions in product manual for optimum PCR results.
- Do not use a kit after its expiration date. Kit components from different lots should not be mixed.

Product Description

Multiplex Real-time PCR is technically able to detect many types of microorganisms simultaneously on many different types of environments. Sensitivity, the specification of this method is quite high, the time of detection is short, it is useful in detecting early hospital infections.

RevoDx CT/NG plus Mycoplasma Hominis qPCR assay is a fluorogenic probe-based PCR assay in which, situated between two PCR primers, there is an internal oligonucleotide probe with a fluorescent label attached at the 5'-end and a quenching molecule that suppresses the fluorescent reporter at the 3'-end. During DNA replication in the PCR process, the internal oligonucleotide hybridizes to the template and is digested by the 5'-3' endonuclease activity of the Thermus aquaticus (Taq) DNA polymerase as the PCR primer is extended. The internal oligonucleotide is digested only if DNA replication occurs, separating the fluorescent and quencher molecules. PCR products are detected within minutes by monitoring the increase in fluorescence that occurs exponentially with successive PCR amplification cycles. The parameter Ct (threshold cycle) is defined as the fractional cycle number at which the fluorescence passes the fixed threshold. A plot of the log of initial target copy number for a set of standards versus Ct is a straight line.

Instruments

The RevoDx CT/NG plus Mycoplasma Hominis qPCR Kit is to be used with BIO-RAD CFX96 Real-Time PCR Detection Systems. But the RevoDx CT/NG plus Mycoplasma Hominis qPCR Kit may also be compatible with most real-time PCR detection systems with the channels FAM, HEX, ROX and Cy5.

General Description

Chlamydia trachomatis is the most common bacterial cause of sexually transmitted genital infections. Most affected individuals are asymptomatic and thus provide a constant reservoir for infection. Conjunctivitis and pneumonia may occur in infants born to mothers through an infected birth canal. Also, both men and women may experience clinical syndromes due to infection in common epithelial sites, including the rectum and conjunctiva. Other types of C. trachomatis infection can occur in both men and women, including lymphogranuloma venereum, an ocular infection that spreads by direct contact and is common in developing countries, and endemic trachoma. Symptoms such as painful urination, vaginal discharge in women, discharge from the penis in men, painful sexual intercourse in women, bleeding between menstrual periods and after sexual intercourse in women, testicular pain in men are seen.

Gonorrhea, or infection with the gram-negative coccus Neisseria gonorrhoeae, is a major cause of morbidity among sexually active individuals worldwide. Like other sexually transmitted infections (STIs), gonorrhea disproportionately impacts young adult populations. There are genital symptoms such as discharge, burning during urination, unusual sores, or rash. N. gonorrhoeae infects the mucous membranes of the reproductive tract, including the cervix, uterus, and fallopian tubes in women, and the urethra in women and men. It is spread during vaginal, anal, or oral sex. A pregnant woman may also pass the infection to her baby during delivery. Anyone who has gonorrhea can pass it on, even without symptoms.

Mycoplasma hominis is a common mollicute bacteria, present in almost all humans in the urinary tract. However, it can sometimes cause infection which can be transmitted sexually. It is different from other STIs, in that monogamous couples can suddenly experience mycoplasma hominis even after years of exclusivity. Mycoplasma hominis is transferred from one person to another by having sex with an infected person and can also be spread from an infected pregnant mother to the baby during delivery. It may also be the culprit behind developing pelvic inflammatory disease (PID) in women. Mycoplasma hominis can also increase the risk of contracting HIV infection if having sexual intercourse with an infected person and may promote a shorter time period before the development of AIDS symptoms.

Safety Information

- Clinical specimens should be treated as potentially infectious; they should be handled in Bio-safety Level 1 or Bio-safety Level 2 area, depending on the infective agents.
- All resulting waste should be considered potentially infectious. They should be handled and discarded according to local safety regulations.
- Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water.
- Avoid producing spills or aerosol.
- Never pipette solutions by mouth
- Do not eat, drink or smoke in laboratory work areas.
- Wash hands after handling samples and test reagents.
- All MSDS information is available upon request
- When working, always wear a protective lab coat, disposable gloves and protective goggles.
- Before and after procedure, disinfect all work surfaces thoroughly with a freshly prepared solution of 10% bleach or antiviral agents.
- Make sure everything is DNase/RNase-free when handling this system.
- Handle all materials according to Good Laboratory Practices in order to prevent cross-contamination.
- Use only calibrated pipettes, always change pipette tips between liquid transfers (aerosol-barrier pipette tips recommended)
- Keep the kit away from any source of contaminating nucleic acids, especially amplified nucleic acid.
- The operations should ideally be done in three separate areas. (i.e. for DNA/RNA purification, PCR setup, amplification) to prevent contamination.
- All equipment and consumables for a particular operation should be kept in the area where that operation is done and should not be moved between separated areas. Gloves should be removed and disposed of before leaving one area to proceed to the next. Lab coats should be specific to each area and never be worn outside the area.
- The work should flow in one direction, beginning in the extraction area followed by the chosen downstream application areas.

Performance Data

Limit of Detection (LoD) - Analytical Sensitivity Study:

To determine the limit of detections (LoD), a dilution series of each pathogen was prepared to give the final concentrations of 2430, 810, 270, 90 and 30 CFU/mL by. spiking urine samples collected from negative individuals to mimic clinical specimens. Pathogen DNA was purified using RevoDx Pathogen DNA/RNA Purification Kit. Each dilution was tested in 24 replicates. The Limit of Detection (LoD) value was calculated by probit analysis. The Limit of Detection (LoD) value was 114 CFU/mL, This LoD value was confirmed by testing an additional 20 replicates spiked at 114 CFU/mL. All 20 replicates produced the positive results for each target, and the LoD was therefore confirmed to be 114 CFU/mL.

Inclusivity:

An *in silico* inclusivity analysis of the RevoDx CT/NG plus Mycoplasma Hominis qPCR Kit primers and probes was performed for the sequences of each pathogen available from NCBI databases. The alignments demonstrated that the regions recognized by the designed primers and probes have 100% homology with all available pathogen sequences from the National Center for Biotechnology Information (NCBI) databases/databanks.

Cross Reactivity:

Cross-reactivity of the RevoDx CT/NG plus Mycoplasma Hominis qPCR Kit was evaluated using both in silico analysis and by wet testing. The in silico analysis of the RevoDx CT/NG plus Mycoplasma Hominis qPCR Kit primers and probes against the sequences of 28 pathogens showed the kit would be specific to the specific targets and not cross-react with these pathogens. The 28 pathogens listed below were wet tested with the RevoDx CT/NG plus Mycoplasma Hominis qPCR Kit for cross-reactivity. No false positive results were observed.
The results from the cross-reactivity, both in silico and wet testing, are summarized below.

***in silico* Cross Reactivity Analysis**

Organism	Result
Pseudomonas aeruginosa	No homology
Klebsiella pneumonia	No homology
Enterobacter cloacae	No homology
Acinetobacter baumannii	No homology
Stenotrophomonas maltophilia	No homology
Staphyloccocus aureus	No homology
Enterococcus faecalis	No homology
Enterococcus faecium	No homology
Bacillus subtilis	No homology
Chlamydia pneumoniae	No homology
Legionella pneumophila	No homology
Streptococcus salivarius	No homology
Streptococcus pyogenes	No homology
Bordetella pertussis	No homology
Mycoplasma pneumoniae	No homology
Pneumocystis jirovecii (PJP)	No homology
Enterococcus dispar	No homology
Proteus spp.	No homology
Saccharomyces cerevisiae	No homology
Schizosaccharomyces pombe	No homology
Aspergillus niger	No homology
Salmonella spp.	No homology
Serratia marcescens	No homology
Parainfluenza virus 1-4	No homology
Influenza A & B	No homology
Respiratory syncytial virus	No homology
Adenovirus (e.g. C1 Ad. 71)	No homology
Human Metapneumovirus (hMPV)	No homology

Wet Tested Cross Reactivity Analysis

Organism	Source	Concentration	Result
Pseudomonas aeruginosa	Clinical specimen	no unitage assigned	Not Detected
Klebsiella pneumonia	Clinical specimen	no unitage assigned	Not Detected
Enterobacter cloacae	Clinical specimen	no unitage assigned	Not Detected
Acinetobacter baumannii	Clinical specimen	no unitage assigned	Not Detected
Stenotrophomonas maltophilia	Clinical specimen	no unitage assigned	Not Detected
Staphyloccocus aureus	Clinical specimen	no unitage assigned	Not Detected
Enterococcus faecalis	Clinical specimen	no unitage assigned	Not Detected
Enterococcus faecium	Clinical specimen	no unitage assigned	Not Detected
Chlamydia pneumoniae	Clinical specimen	no unitage assigned	Not Detected
Legionella pneumophila	Clinical specimen	no unitage assigned	Not Detected
Streptococcus pyogenes	Clinical specimen	no unitage assigned	Not Detected
Bordetella pertussis	Clinical specimen	no unitage assigned	Not Detected
Mycoplasma pneumoniae	Clinical specimen	no unitage assigned	Not Detected
Pneumocystis jirovecii (PJP)	Clinical specimen	no unitage assigned	Not Detected
Enterococcus dispar	Clinical specimen	no unitage assigned	Not Detected
Aspergillus niger	Clinical specimen	no unitage assigned	Not Detected
Human coronavirus (229E)	NIBSC (Cat. No: 09/132)	no unitage assigned	Not Detected
Human Adenovirus	NIBSC (Cat. No: 16/324)	2.0×10^{8} IU/ml	Not Detected
Influenza Virus (A/Christchurch/1/2003, H1N1)	NIBSC (Cat. No: 07/296)	no unitage assigned	Not Detected
Influenza Virus (A/Wyoming/3/2003, H3N2)	NIBSC (Cat. No: 07/298)	no unitage assigned	Not Detected
Influenza Virus (B/Jiangsu/10/2003)	NIBSC (Cat. No: 07/300)	no unitage assigned	Not Detected
Human Immunodeficiency Virus 1 (HIV-1)	NIBSC (Cat. No: 16/194)	1.25×10^{5} IU/ml	Not Detected
Human Immunodeficiency Virus 2 (HIV-2)	NIBSC (Cat. No: 16/296)	2.8×10^{5} IU/ml	Not Detected
Human Respiratory syncytial virus A2	NIBSC (Cat. No: 08/120)	no unitage assigned	Not Detected
Parainfluenza virus type 1	NIBSC (Cat. No: 08/176)	no unitage assigned	Not Detected
Parainfluenza virus type 2	NIBSC (Cat. No: 08/178)	no unitage assigned	Not Detected
Parainfluenza virus type 3	NIBSC (Cat. No: 08/118)	no unitage assigned	Not Detected
Parainfluenza virus type 4	NIBSC (Cat. No: 08/180)	no unitage assigned	Not Detected

Clinical Evaluation:

The performance of the RevoDx CT/NG plus Mycoplasma Hominis qPCR Kit was evaluated using archived urine samples or urogenital swabs or cervical swabs. For each pathogen, a total of 20 positive and 20 negative specimens were tested in a randomized and blinded fashion. All the 20 positive specimens and the 20 negative specimens were collected from a state hospital lab and had previously been tested with a validated comparator assay. Samples were extracted by RevoDx Pathogen DNA/RNA Purification Kit according to the product manual. Then, PCR reactions were setup by RevoDx CT/NG plus Mycoplasma Hominis qPCR Kit according to the product manual. BIO-RAD CFX96 Real-Time PCR Detection System was used for amplification, detection and analysis.

According to the test results, 100% agreement was observed with expected results.

Additional Materials Required

- RevoDx Pathogen DNA/RNA Purification Kit (Cat. No: IP202302; idil biotech,Turkey) or RevoDx Magnetic Pathogen DNA/RNA Purification Kit (Cat. No: IP202303; idil biotech,Turkey) or DirEXT OneStep Pathogen DNA/RNA Extraction Reagent (Cat. No: IP202319; idil biotech,Turkey)
- Real-Time PCR Detection System,
- Suitable protection (protective lab coat, disposable gloves, protective goggles, etc.)
- Micropipettes (0.5 µl – 1000 µl),
- DNase/RNase-free micropipette tips with filters,
- DNase/RNase-free 1.5 ml microcentrifuge tubes,
- Vortex mixer,
- Desktop microcentrifuge for PCR plates/strip tubes,
- PCR Workstation,
- Real-Time PCR reaction tubes or plates,

Sample Preparation

This kit has been validated for use with urine samples or urogenital swabs or cervical swabs. Furthermore, all nucleic acid samples that are suitable for qPCR assays can be used with RevoDx CT/NG plus Mycoplasma Hominis qPCR Kit. Clinical specimens should be treated as potentially infectious; and the precautions are recommended during sample collection and handling.
Clinicians (including healthcare assistants, nurses, doctors and professionals allied to medicine) have the responsibility of using the correct procedure during the collection and safe transportation of samples to the laboratory. The validity of test results largely depends on good practice in the 'pre-test' stage and it is essential that documentation is accurate and comprehensive.
After collecting, do not store the specimens at room temperature for longer than 4 hours. Transportation of the specimens must conform to country or local regulations.

Protocol

DNA Extraction: RevoDx Pathogen DNA/RNA Purification Kit or RevoDx Magnetic Pathogen DNA/RNA Purification Kit or DirEXT OneStep Pathogen DNA/RNA Extraction Reagent should be used for pathogen DNA extraction from urine samples or urogenital swabs or cervical swabs. Please follow the manufacturer's instructions as stated in the kit manual. The operations should ideally be done in three separate areas. (i.e. for DNA/RNA purification, PCR setup, amplification) to prevent contamination.

Internal Control: An internal (Hs_RPP30) control targeting RNase P is needed to verify that nucleic acid is present in every sample and is used for every sample processed. This also serves as the extraction control to ensure that samples resulting as negative contain nucleic acid for testing.

Positive Control: To be able to evaluate the experiment, the Ct values of Positive Control should be equal to 25 ± 4, otherwise, it indicates a problem.

PCR Protocol

1. Thaw all components at room temperature except STI-31 Enzyme Mix. Put STI-31 Enzyme Mix on ice. Mix each component thoroughly, then centrifuge briefly before use. Transfer all the reagents onto ice or cooling block.

2. The final volume of Master Mix is obtained by multiplying single reaction volumes of STI-31 MM and STI-31 Enzyme Mix by the total sample size. When calculating the total sample size, the number of negative controls, positive controls and the clinical samples should be taken into consideration. For possible pipetting errors, it is recommended to add an extra sample to the total sample size.

3. To prepare master mix, add 14 µl of STI-31 1 and 1 µl of STI-31 RM 2 for each sample to the master mix tube. Vortex the tube and spin down briefly in a microcentrifuge. Add 15 µl of Master Mix into Real-Time PCR reaction tubes or capillaries for each sample. Add 5 µl DNA of each sample, negative control and positive control into the tubes. Spin down briefly.

4. Enter cycling conditions for Real-Time PCR Detection System: 95°C for 2 min, 1 cycle; 95°C for 10 sec, 60°C for 20 sec, 40 cycles (Table 1). Enter 20 µl as sample volume.

Table 1: Amplification program

Program Name	Cycles	Program
Hot Start	1	95ºC, 2 min
Amplification*	40	95ºC, 10 sec
		60ºC, 20 sec

*** Fluorogenic data should be collected at 60°C; FAM, HEX, ROX and Cy 5 channels should be chosen**

5. Fluorogenic data is collected at 60°C. FAM, HEX, ROX and Cy 5 channels should be selected.

6. Start run.

7. To program and analyze the results, refer to the User Manual of the instrument concerned.

Data Analysis

In order to evaluate the assay, the Ct value of Positive Control must be equal to 25±4 for FAM, HEX and ROX dyes and Negative Control in all channels must be negative. Otherwise, the experiment should be repeated.

The results can be interpreted as:

Signal in any FAM channel (Chlamydia trachomatis DNA)	Signal in any HEX channel (Neisseria gonorrhoeae DNA)	Signal in any ROX channel (Mycoplasma Hominis DNA)	Signal in Cy5 channel (Internal Control)	Interpretation
+	-	-	+/-	Chlamydia trachomatis DNA is positive
-	+	-	+/-	Neisseria gonorrhoeae DNA is positive
-	-	+	+/-	Mycoplasma Hominis DNA is positive
-	-	-	+	Target DNA is not detected
-	-	-	-	Invalid result. This sample should be re-tested

Ordering Information

Product Name	Package	Cat. No.
RevoDx CT/NG plus Mycoplasma Hominis qPCR Kit	50 tests	IP202415-50
RevoDx CT/NG plus Mycoplasma Hominis qPCR Kit	100 tests	IP202415-100
RevoDx CT/NG plus Mycoplasma Hominis qPCR Kit	500 tests	IP202415-500