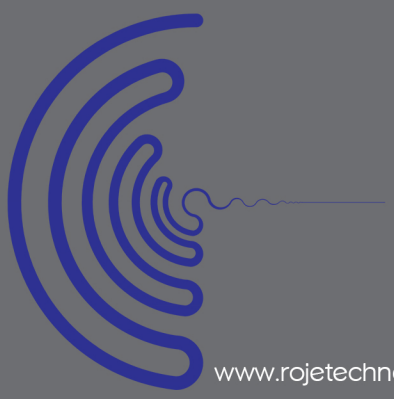

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ROJETECHNOLOGIES

Viga MTB Molecular Diagnostic Kit

Qualitative Real Time-PCR Assay

Molecular detection MTB kit based on Real Time-PCR

For In Vitro Diagnostic Use

By ROJE

Edition, 01/2022

ROJETechnologies has been founded since 2014, and manufactures a wide range of molecular biology kits. We research, develop and create our products in order to make easier and more comfortable approaches to do research in molecular biology. Our target is offering high-quality, affordable Molecular and diagnostic Kits and reagents comparable to the world leaders to research centers, laboratories, clinics, hospitals, and diagnostic centers all over the world.

Contents

Kit content

Kit content	25 Preps	100 Preps
Pro MTB Mix	250µl	1000µl
QR-ROMAX, 4X	125µl	500µl
MTB Positive Control	40µl	150µl
RT-PCR Grade Water	40µl	150µl

Storage

ROJETechnologies checked Shipment condition. After arrival, all reagents should be kept in darkness, at -25℃ to -20℃ temperature. Do not freeze-thaw the Kits frequently. When storage condition is as directed, all reagents are stable until the expiration date, as indicated on the kit box.

Intended Use

Viga MTB molecular diagnostic Kit Real Time-PCR Kit is an in vitro diagnostic kit designed to detect Mycobacterium tuberculosis (MTB) DNA in human samples such as sputum, bronchoalveolar lavage (BAL), bronchial secretion, CSF, stomach fluid or peritoneal punction and urine through Real Time-PCR.

Guarantee and Warranty

ROJETechnologies guarantee the efficiency of all manufactured kits and reagents. For more information on choosing proper kits based on your needs, please contact our technical support team. If any of the products do not meet your satisfaction, please contact our technical support team due to reasons other than misuse. If the problem is due to the manufacturing process, the ROJE team will replace the kit for you.

Notice to Purchaser

This product is only for experimental and not for commercial use of any kind. There is no right to resell the kit or any of its components. For information about our licensing or distributors, contact the ROJE business team.

Warning and Precautions

Material Safety Data Sheet (MSDS) for all products and reagents are provided. They are accessible online at www.rojetechnologies.com.

Dear users, please follow laboratory safety rules.

Please study the guideline precisely before use.

You must consider all samples of patients and positive controls potentially infectious.

- Eating, drinking, smoking, chewing gum, applying cosmetics, and taking medicine in laboratories where hazardous materials and human samples are used should be prohibited. Consider all samples of patients and positive control potentially infectious.
- The physician's prescription uses the Viga MTB molecular diagnostic Kit for emergency and in Vitro diagnostic use.
- Each step of procedures such as sampling, storage, shipping, and laboratory tests must follow biosafety and molecular laboratory management.
- Due to this test, separate and private laboratory space is needed:

Place 1: preparation area- the components of test preparation.

Place 2: sample processing- isolation and controlling are done.

Place 3: amplification area where Real Time-PCR test is done.

- The clinical laboratories must be equipped with instruments and operators following the rules of the Ministry of Health.
- All kit contents have been developed to test MTB; changing or replacing any kit content will affect its function, in contrast with the product license.
- All pipette tips and microtubes must be sterile and DNase-RNase-free. In order to avoid contamination, filter pipette tips are needed, and they must be changed after adding any substance or samples.
- Landfill the waste according to biosafety guidelines. All desks and laboratory instruments must be antisepticised regularly with 70% Ethanol or 10% Sodium Hypochlorite.
- Avoid putting the combination of Pro MTB in exposure to sunlight.

Quality control

According to clinical and laboratory standards, institute, and WHO, Viga MTB molecular diagnostic Kit is tested against predetermined experiments on a lot-to-lot basis to ensure consistent product quality. For your information, the results of all experiments are accessible by addressing REF and Lot number on the web at www.rojetechnoloes.com

Equipment & Reagents to Be Supplied by User

- DNase-RNase-free microtubes (1.5ml)
- PCR microtube 0.1ml or 0.2ml strip
- Various models of pipette and pipette tips (10µl, 100µl, and 1000µl of filter pipette tips)
- Surface sanitizing solution like RNZO (Cat No: RN983048)
- Disposable Powder- Free gloves and surgical gown
- Different types of Real Time-PCR Instruments (with green, yellow channels)
- Centrifuge (can reach 13000 rpm)
- Microcentrifuge
- Vortex
- Cool box

Procedures

Tuberculosis is an airborne mycobacterial infection caused by the M. tuberculosis complex (M. tuberculosis, M. africanum, M. bovis, M. bovis BCG, M. microtia, M. pinnipedii). MTBC is spread from one person to another through tiny droplets released into the air via coughs and sneezes. Early detection of TB is essential to further improve health outcomes for people with TB and reduce TB transmission more effectively. Viga MTB molecular diagnostic kit testifies a polymerase chain reaction of Real Time-PCR. This kit is designed for qualitative diagnosis of IS6110 gene (specific multi-copy insertion sequence) Mycobacterium Tuberculosis. After nucleic acid isolation by using the DNJia Tissue and Bacteria Kit or other kits that are approved by the Ministry of Health and verified sample combination could be added to the master mix primer/probe mix to perform the reaction. In addition, the Viga MTB molecular diagnostic kit contains a second heterologous amplification system to identify possible PCR inhibition. This is detected as an internal control (IC) in fluorescence channel Cycling Yellow of the Rotor-Gene Q MDx, Rotor-Gene Q, or Rotor-Gene 6000, or Cycling A.JOE of the Rotor-Gene 3000. With the help of its sampling,

the quality of sample isolation and reaction process of PCR can be checked and controlled to prevent false-negative results. The results demonstrated that the LoD of an assay is 25 Copies/ml.

Applications

Viga MTB molecular diagnostic kit is an in vitro nucleic acid amplification test for the detection of all members of the *M. tuberculosis complex (M. tuberculosis, M. africanum, M. bovis, M. bovis BCG, M. microti, M. pinnipedii)* in human sputum, BAL, bronchial secretion, CSF, stomach fluid or peritoneal punction samples. This diagnostic test kit utilizes the polymerase chain reaction (PCR) and is configured with Real Time-PCR instruments.

Features

Technology	Real Time-PCR
Type of Analysis	qualitative
Target Sequence	IS6110 genes (specific multi-copy insertion sequence)
Analytical Specificity	Mycobacterium tuberculosis complex (M. tuberculosis, M. bovis, M. africanum, M. microti, M. caprae, M. canetti and vaccine strain BCG), 100%
Analytical Sensitivity (LOD)	LoD of assay is 25 Copies/ml with the probability 95%
Diagnostic Specificity	100% (CI95%: 99.06% –100%)
Diagnostic Sensitivity	100% (CI95%: 99.06% –100%)
Extraction/Inhibition Control	PCR inhibition and DNA extraction efficiency control
Validated Specimen	Human sputum, BAL, bronchial secretion, CSF, stomach fluid or peritoneal punction
Storage	-20 ± 5°C
Validated Extraction Method	DNJia Tissue and Bacteria Kit
Instruments	Rotor-Gene Q, 2plex, Corbett Rotor-Gene 3000&6000, Mic qPCR Cycler, StepOne and StepOne plus Applied Biosystem
Required Detection Channels	Green-Yellow

Recommended Starting Material

- Before starting any tests, each component must be thawed, vortexed, and centrifuged briefly. Avoid repeated freeze-thaw cycles.

Sample Storage and Preparation

Human sputum, BAL, bronchial secretion, CSF, stomach fluid, or peritoneal punction samples

The fresh specimen must either be processed immediately as per the sample procedure outlined in the section on Sample processing protocol or stored frozen at -20℃. Frozen samples must be brought to room temperature before starting sample processing. Sample Pre-treatment decontaminates the specimen and makes it ready for extraction.

Before Start

Take out each component from the kit and place them on the benchtop. Allow the reagents to equilibrate to room temperature, then briefly vortex each tube for later use.

Buffer Preparation

Table 1: preparation of components per single reaction

components	Volume
Q-ROMAX, 4X	5µl
Pro MTB Mix	10µl
Isolated DNA	5µl

Pathogenicity

Mycobacterium tuberculosis is a species of pathogenic bacteria in the family Mycobacteriaceae and the causative agent of tuberculosis. First discovered in 1882 by Robert Koch. According to the most recent Global Tuberculosis Report (2019), edited by the World Health Organization (WHO), TB is considered the ninth cause of death worldwide and the leading cause of mortality by a single infectious agent, with the highest rate of infections and death toll rate mostly concentrated in developing and low-income countries. Human MTB infections usually begin by inhaling aerosol droplets containing tubercle bacilli directly expectorated from an individual with "open" pulmonary disease. The infectious dose for a person is reported to be between 1 and 200 bacilli; however, as a single aerosol droplet can contain anywhere from 1 to 400 bacilli, it is

unclear what is considered a biologically relevant dose. The bacilli travel to the alveoli, where they are rapidly phagocytosed by alveolar macrophages. MTB pathogenicity is mainly based on the capability of the bacilli to reprogram host macrophages after primary infection, preventing its own elimination; the formation of granulomas, in which the pathogen survives in equilibrium with the host defense and the slowing control of bacterial central metabolism and replication, characterizing the so-called dormant state in which MTB is resistant to host defenses and therapy.

Workstation preparation

All work surfaces, pipettes, centrifuges, and other supplies must be cleaned and sanitized before use. To reduce the risk of nucleic acid contamination, use sanitizers like 70% Ethanol or 10% Sodium Hypochlorite.

Protocols

Thaw all reagents thoroughly at room temperature (15–25°C). When thawed, mix all reagents (by repeatedly pipetting up and down or by pulse vortexing) and centrifuge briefly. Work quickly and keep all reagents in the cooling block.

The volume of eluted sample DNA in this test should be 5µl. Prepare PCR reaction and then perform Real Time-PCR**.**

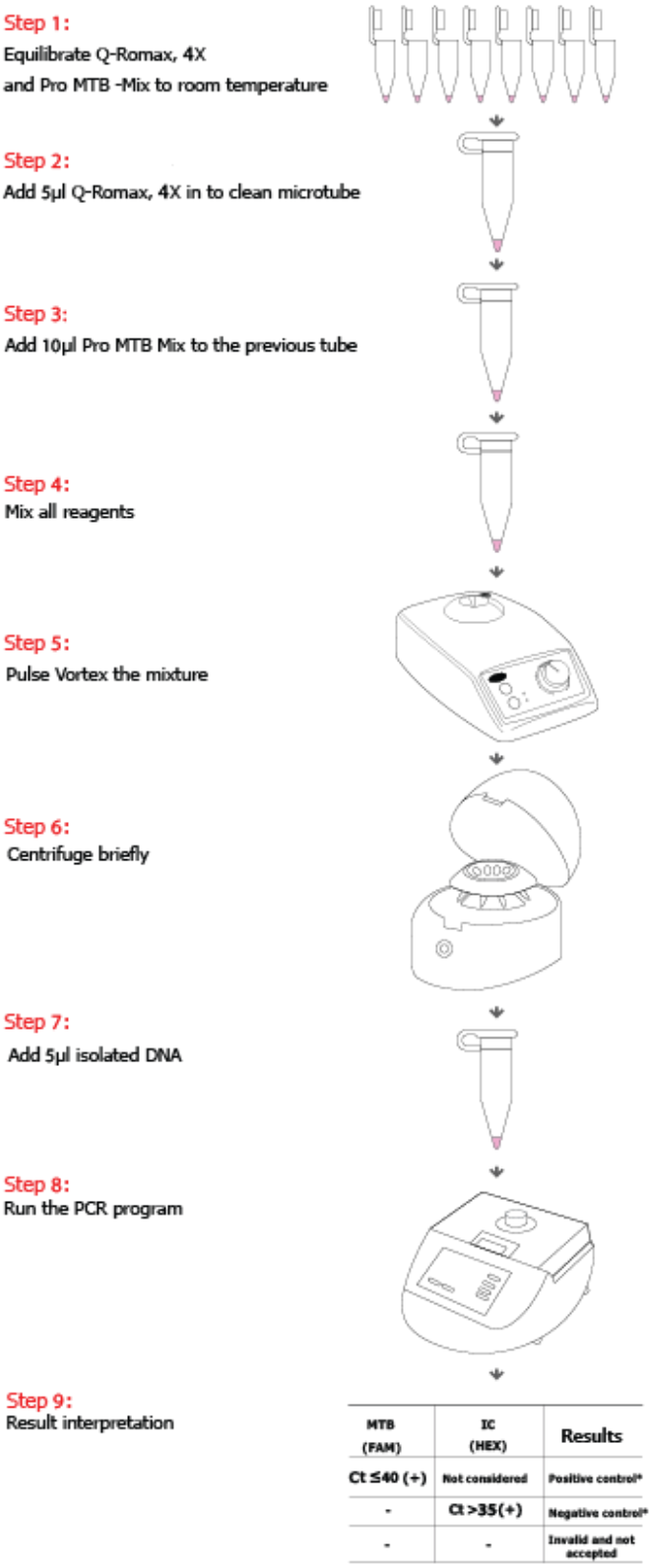

Figure 1: preparation of reagents, PCR run, and interpretation of results

PCR reaction preparation

Table 2: PCR reaction preparation

components	Volume
Q-ROMAX, 4X	5µl
Pro MTB Mix	10µl
Isolated DNA	5µl

Thermal Profile

Table 3: Thermal profile for PCR reaction

Stage	Temperature	Incubation Time	Cycle Numbers
Pre-Denaturation	95°C	3 min	1
Denaturation	95°C	10 sec	45
Annealing and acquisition on channels Green and Yellow	60°C	40 sec	

Results Interpretation

- Data analysis for each gene should be performed separately by using a manual threshold.
- The threshold for each sample should be in the exponential phase of the fluorescence curves and above any background signal.
- FAM Fluorophore (green) for the IS6110 gene of *M. tuberculosis*, and HEX Fluorophore (Yellow) is for the IC gene (internal control).
- A negative control is used as contamination control. The magnitude increase of the Fluorescence curve in the negative control does not cross the threshold. If Ct is less than 35 (Ct<35), it is considered a possible contamination. Strong signals above 35 in

the NTC can be PCR artifacts, and in these cases, the shape of the curve can be considered (the S-shaped curve is typical for a positive result).

- Internal control should be positive for all clinical specimens at Ct 35 or less than 35, indicating sufficient nucleic acid from the human gene and the sample has acceptable quality.
- Internal control curve Ct>33 or without Ct indicates low sample concentration or inhibitors in the reaction (the isolated sample is recommended to dilute at least ½). If the test result is not acceptable again during the retest, another new sample should be taken from the patient, and the test must be repeated.
- A positive clinical specimen should have Ct≤40 for the target gene (IS6110).
- If the expected positive reaction is not achieved (typical S-shaped curve), the performed test is not acceptable, and the test must be repeated by following the kit instructions accurately.
- Determine the reason for the failure of positive control, take the corrective action, and document corrective action results.

The acceptable situation for positive and negative control

Table 4: Control conditions for a valid PCR Run

Results	IC (HEX)	MTB (FAM)
Positive control*	Not considered	Ct≤40 (+)
Negative control	Ct>35 (+)	-
Invalid and not accepted	-	-

Test limitations

- A false-negative result is obtained in case of low titration of MTB in the patient sample, improper transportation, and lack of quality of sample isolation.
- All controls must be checked before the interpretation of the results. If the controls are not valid, the patient's results cannot be interpreted. The diagnostic limit of this kit equals

Ct≤40, and also, the user must review the fluorescence curve before final interpretation. All positive curves must have an amplification peak.

- Non-observance of proper storage conditions of the kit can lead to false-negative results.
- Handling this kit needs experienced and trained personnel. Any personnel error may lead to invalid results.
- The results of this diagnostic kit are only acceptable if they are along with clinical evidence for diagnosing MTB. Definitive diagnosis and treatment of patients must be based on a combination of this test with other test results, medical records, and how to respond to treatment.

Evaluation and Qualification Analysis

Preparation of a standard sample

- DNA isolation was performed from a sample infected with MTB (10000 Copies per ml). From the same sample, serial dilution of 10000, 1000, and 100 Copies per ml was prepared, and an average of ten repetitions was set for each dilution using the Artus M. tuberculosis RG PCR Kit (Qiagen).

Limit of Detection (LoD)-Analytical sensitivity

LoD studies were used to determine the lowest detectable concentration of MTB DNA, at which approximately 95% of all (true positive) replicates test positive. The LoD was determined by limiting dilution studies using characterized samples.

The analytical sensitivity in consideration of the purification (DNJia Tissue and Bacteria Kit) of the Viga MTB molecular diagnostic kit was determined using dilution series of the plasmid standards from 50 to nominal 12.5 MTB Copies/ml spiked in clinical sputum specimens.

The LoD of each test was then confirmed by testing 20 replicates with dilution series (50, 25, 12.5 Copies/ml) at the tentative limit of detection. The final LoD of each test was determined to be the lowest dilution series resulting in positive detection of 19 out of 20 replicates.

The LoD of the Viga MTB molecular diagnostic kit was established using DNJia Tissue and Bacteria Kit. The results demonstrated that the LoD of an assay is 25 Copies/ml.

Detection Results of Viga MTB molecular diagnostic kit Using DNJia Tissue and Bacteria Kit.

Table 5: Determination of Viga MTB molecular diagnostic kit based on DNJia Tissue and Bacteria Kit

Test No	(Copies/mL)		
	MTB		
	50	25	12.5
1	35.17	35.28	36.50
2	34.30	35.06	36.57
3	33.61	37.35	35.49
4	35.02	37.49	Undetermined
5	34.49	36.13	Undetermined
6	34.07	36.02	Undetermined
7	34.41	35.49	37.04
8	34.37	36.60	Undetermined
9	34.53	35.42	38.58
10	34.89	35.61	37.53
11	35.06	37.62	Undetermined
12	34.36	36.46	38.91
13	34.28	38.48	36.28
14	33.92	36.66	37.60
15	34.76	36.15	Undetermined
16	34.76	35.91	Undetermined
17	35.72	37.46	Undetermined
18	38.22	35.19	37.41
19	34.04	38.06	38.65
20	36.19	36.25	Undetermined

Positive percentage in each concentration	100%	100%	55%

Analytical specificity

Inclusivity of the primer/probe set used in the Viga MTB molecular diagnostic kit was analyzed in silico based on MTB sequences from the NCBI database accessed on September 26, 2021. The primer/probe sets for IS6110 gene sequence alignment analysis demonstrate 100 inclusivity for MTB sequences identified from patient samples. The representative alignment results for the IS6110 gene are shown in the table.

Table 6: Alignment test result for IS6110 gene

Strain	Target	Accession	% Homology Test Forward primer%	% Homology Test Reverse primer%	% Homology Test Probe%
Mycobacterium tuberculosis strain 2.2	IS6110	CP074075.1.1	100	100	100
Mycobacterium tuberculosis R2092 DNA	IS6110	AP024671.1.1	100	100	100
Mycobacterium tuberculosis strain H37Rv_CG	IS6110	CP072765.1.1	100	100	100
Mycobacterium tuberculosis strain CG24	IS6110	CP072761.1.1	100	100	100
Mycobacterium tuberculosis strain CG21	IS6110	CP072763.1	100	100	100
Mycobacterium tuberculosis strain CG20	IS6110	CP072764.1	100	100	100
Mycobacterium tuberculosis strain CG23	IS6110	CP072762.1	100	100	100
Mycobacterium tuberculosis strain 267/47W148	IS6110	CP071128.1	100	100	100
Mycobacterium tuberculosis strain 120/26CAO	IS6110	CP071127.1	100	100	100
Mycobacterium tuberculosis strain 11502	IS6110	CP070338.1	100	100	100
Mycobacterium tuberculosis variant microti strain Mycobacterium microti 94-2272	IS6110	LR882500.1	100	100	100

Mycobacterium tuberculosis variant microti OV254	IS6110	LR882499.1	100	100	100
Mycobacterium tuberculosis variant microti strain Mycobacterium microti Maus III	IS6110	LR882498.1	100	100	100
Mycobacterium tuberculosis variant microti strain Mycobacterium microti Maus IV	IS6110	LR882497.1	100	100	100
Mycobacterium tuberculosis variant microti strain Mycobacterium microti ATCC 35782	IS6110	LR882496.1	100	100	100
Mycobacterium orygis strain MUHC/MB/EPTB/Orygis/51145	IS6110	CP063804.1	100	100	**100**
Mycobacterium tuberculosis strain 1-0006P6C4	IS6110	CP041876.1	100	100	100
Mycobacterium tuberculosis strain 2.2	IS6110	CP074075.1.1	100	100	100
Mycobacterium tuberculosis R2092 DNA	IS6110	AP024671.1.1	100	100	100

Clinical sensitivity

The wet testing of inclusivity using the DNJia Tissue and Bacteria Kit was evaluated as supplemental data by testing three MTB positive specimens. These specimens were confirmed positive by Viga MTB molecular diagnostic kit. Each specimen was diluted to (≤3log10 LOD, ≤2log10 LOD, ≤1log10 in the negative specimen matrix (Sputum specimen) and tested in the tenth replicate. (Below table).

Table 7: Clinical sensitivity of Viga MTB molecular diagnostic kit (ROJETechnologies)

Dilution series	IU/ml	Ct
≤3log10 LOD	25,000	26.64
		27.27
		27.05
		26.63
		26.71
		26.27
		26.48
		26.59
		27.44
		26.37
≤2log10 LOD	2,500	32.84
		31.88

		33.31
		33.60
		32.78
		31.63
		32.68
		32.17
		34.08
		32.87
≤1log10 LOD	250	36.54
		36.82
		34.62
		34.78
		35.58
		35.61
		35.44
		34.76
		35.78
		35.20

Cross-reactivity (analytical specificity)

The Cross-reactivity of the Viga MTB molecular diagnostic kit was evaluated both in silico analysis and by wet testing potentially cross-reactive whole pathogens or purified nucleic acid from clinical specimens. No cross-reactivity was detected. The in-silico mapping analysis of each primer/probe against several pathogens is based on the NCBI nr/nt database accessed September 28, 2021, using the online BLASTN 2.10.0+, and the representative results are shown below the table. No cross-reactivity was observed for other listed respiratory borne- pathogens based on both in silico and wet-testing.

Table 8: The In-Silico Specificity Analysis of Primer and Probe Set for Other respiratory-borne pathogens.

Pathogen (Taxonomy ID)	**Strain**	**GenBank Acc#**	**% Homology Test FP**	**% Homology Test RP**	**% Homology Test Probe**
Human coronavirus	HCoV_OC43/Seattle/USA/SC0776/2019	MN310478.1	52	85	**50**
Human respiratory syncytial virus A	RSVA/Homo sapiens/USA/MCRSV_211/1980	MG642060.1	47	50	**44**

Human respiratory syncytial virus B	RSVB/Homo sapiens/USA/MCRSV_267/1983	MG642059.1	42	50	**58**
Human adenovirus	HAdV7/China/Hubei/19S0082726/2019-06-07	MW816101.1	88	75	**80**
Haemophilus influenzae	P602-8883	CP033168.1	72	55	**50**
Human Metapneumovirus (hMPV)	A/NSW/WM2014916/16	MW221986.1	47	65	**44**
Parainfluenza virus 1-4	HPIV3/38/ZJ/CHN/2018	MN145876.1	44	50	**60**
Rhinovirus	JC201/Zhuhai/GD/CHN/2013	KM613168.1	57	55	**61**
Chlamydia pneumoniae	LPCoLN	CP001713.1	66	60	**55**
Legionella pneumophila	ST42	LT632617.1	77	95	**100**
Streptococcus pneumoniae	2245STDY5982722	LR216031.1	57	65	**72**
Streptococcus pyogenes	FDAARGOS_668	CP044093.1	66	80	**66**
Bordetella pertussis	J029	CP046995.1	90	72	**50**
Mycoplasma pneumoniae	463 satellite Mpn16	MW920166.1	55	95	**52**
Candida albicans	TIMM 1768	CP032016.1	66	70	**55**
Pseudomonas aeruginosa	CF39S	CP045917.1	66	100	**65**
Staphylococcus epidermis	NW32	KT726221.1	57	90	**55**
CMV	IRNToG3	KC122248.1	55	50	**55**
Cryptococcus neoformans	H99 CMGC/DYRK/DYRK2	XM_012196141.1	72	65	**66**
human genome	AKR1C3	NG_047094.1	56	78	**60**

Cross-reactivity (clinical specificity)

To check the clinical specificity of the nucleic acid of respiratory pathogens in a matrix of a negative sample (negative sputum) diluted with a certain concentration. Then the samples were extracted and, using Viga MTB molecular diagnostic kit was tested. No cross-reactivity was observed for other listed respiratory pathogens in the following table.

Table 9: Investigation of the cross-reactivity of the MTB using Viga MTB molecular diagnostic kit.

Virus/Bacteria/Parasite	Source/ Sample type	Ct Value
Adenovirus	AmpliRun®DNA/RNA Vircell	**-/-**
Influenza A	AmpliRun®DNA/RNA Vircell	**-/-**
Influenza B	AmpliRun®DNA/RNA Vircell	**-/-**
Legionella pneumophila	AmpliRun®DNA/RNA Vircell	**-/-**
Cryptococcus neoformans	AmpliRun®DNA/RNA Vircell	**-/-**
Chlamydia pneumonia	AmpliRun®DNA/RNA Vircell	**-/-**
Streptococcus pneumoniae	AmpliRun®DNA/RNA Vircell	**-/-**
Respiratory Syncytial Virus	AmpliRun®DNA/RNA Vircell	**-/-**
Mycoplasma pneumoniae	AmpliRun®DNA/RNA Vircell	**-/-**
Streptococcus pyogenes	AmpliRun®DNA/RNA Vircell	**-/-**
Mycobacterium tuberculosis	AmpliRun®DNA/RNA Vircell	**-/-**
10 Pooled human genomes	Clinical sample	**-/-**

Precision

assessment includes In Vitro Intra-assay and Inter-assay.

Intra-assay

Intra-assay refers to the precision and ability of the designed method in determining the concentration of similar repeats in one Real Time-PCR run, which as Mean±SD for different Cts is shown. For this purpose, three repetitions of each concentration of the control sample were examined in one reaction, and coefficient of variation values was calculated for the threshold cycle values. The result of the Run for the target gene (IS6110) in the maximum coefficient of variation is 1.17, and the minimum of the coefficient of variation is 0.7. All acceptable results must have a CV of less than 5%.

Inter-assay

Inter-assay refers to the results of different Runs in Real Time-PCR or results from other laboratories expressed as Mean±SD for different Cts related to the number of copies or different concentrations of a sample. For this purpose, five repeats of each concentration of the control sample were tested on three additional days. Run result for the target gene (IS6110) in maximum coefficient of variation is 1.78, and the minimum coefficient of variation is 1.09. All acceptable results must have a cv of less than 10%.

Clinical Evaluation

The clinical performance of the Viga MTB molecular diagnostic kit was established using 130 Human sputum, BAL, bronchial secretion, and CSF specimens collected from patients who were suspected of MTB. The comparator method was the Artus M. tuberculosis RG PCR Kit (Qiagen), which received CE-IVD. The extraction method was the DNJia Tissue and Bacteria Kit. Both assays were run on Rotor Q (Qiagen). The results are summarized in the analysis part and demonstrated a PPA of 100% and NPA of 100%.

Clinical assessment between Viga MTB Molecular Diagnostic kit (ROJETechnologies) and Artus M. tuberculosis RG PCR Kit (Qiagen).

Table 10: Clinical Evaluation of Viga MTB Molecular Diagnostic kit

Test		**Artus M. tuberculosis RG PCR Kit (Qiagen)**		**Total**
		Positive	**Negative**	
Viga MTB molecular diagnostic kit	**Positive**	30	0	30
	Negative	0	100	100
Total		30	100	130

- Positive Agreement Rate: 100/100 ×100%=100%
- Negative Agreement Rate: 100/100×100%= 100%
- Overall rates of agreement: (30+100) / (30+0+100+0) ×100%=100%

symbols

Tables 11: symbols on the Kit Label

symbols	meaning	symbols	meaning
	Date of manufacture		manufacturer
	Expiration Date		Temperature limitation
IVD	In Vitro Diagnostics	LOT	Lot number
		REF	Reference number

Troubleshooting

Here we try to cover as many problems as you may see in using this product; however, scientists in ROJE Technical Support Team are eager to answer all your questions. Do not hesitate to contact us for more information.

Problems	Possible Causes	Action
No fluorescent signal is detected in any samples, including positive control	Error in the preparation of the master mixture	Verify each component and ensure the volumes of reagent dispensed during the preparation of the master mixture are correct. Repeat PCR mixture preparation.
	Instrument settings error	Verify the Real Time-PCR instrument settings are correct.
If the fluorescent signal is detected in a negative control reaction	Contamination of the extraction/preparation area	Clean surfaces and instruments with aqueous detergents, wash lab coats and replace test tubes and tips in use.
	PCR tube not properly sealed	Ensure plates are sealed correctly
If the fluorescent signal does not display the sigmoidal characteristic	Components degraded	Use a new batch.
	Poor quality of DNA samples carrying interferences	Repeat the test with the neat extracted DNA and 1:2 dilution of the extracted DNA.
	PCR equipment failure	Repeat the test or contact the equipment supplier

Ordering Information

category	Product name	Preps no.	Cat No.
Molecular diagnostic Kit	Viga MTB Molecular Diagnostic Kit	25 Preps	MD003057
	Viga MTB Molecular Diagnostic Kit	100 Preps	MD003054

Technical assistance

ROJETechnologies guarantee your complete satisfaction. ROJE technical support team is composed of highly trained, experienced scientists; who can troubleshoot most problems you face. Our technical support team can offer expert advice which may help you select a suitable product.

Contact our technical support at any time by selecting one of these ways:

- Through our telephone and fax number; +982191070705.
- You can submit your question directly to ROJE Technical Support Team from our website (www.ROJETechnologies. com)
- Or send your questions to this email address, technicalsupport@rojetechnologies.com.

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ROJETECHNOLOGIES has been founded since 2014, and manufactures a wide range of molecular biology kits. We research, develop and create our products in order to make easier and more comfortable approaches to do research in molecular biology. Our target is offering high-quality affordable Molecular and diagnostic Kits and reagents, comparable of the world leaders, to research centers, laboratories, clinics, hospitals and diagnostic centers all over the world.

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