Stark MTB Molecular Diagnostic Kit

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Contents

Description

The Stark MTB Molecular Diagnostic Kit sets the benchmark for precise and efficient detection of Mycobacterium tuberculosis (MTB) complex members, including M. tuberculosis, M. africanum, M. bovis, M. bovis BCG, M. microti, and M. pinnipedii. This advanced in vitro nucleic acid amplification test leverages the power of polymerase chain reaction (PCR) technology, specifically configured for Real-Time PCR instruments. By targeting MTB DNA within human sputum, bronchoalveolar lavage (BAL), bronchial secretion, cerebrospinal fluid (CSF), stomach fluid, peritoneal puncture, and urine samples, this Kit provides rapid and accurate identification of MTB complex infections. Its robust design ensures reliable performance, making it an indispensable tool in the diagnosis and management of tuberculosis.

Intended Use

The Stark MTB Molecular Diagnostic Kit Real-Time PCR Kit is intended for in vitro diagnostic use by trained healthcare professionals. Designed for the detection of Mycobacterium tuberculosis (MTB) DNA in human samples, including sputum, bronchoalveolar lavage (BAL), bronchial secretion, cerebrospinal fluid (CSF), stomach fluid, peritoneal puncture, and urine, this Kit employs Real-Time PCR technology for precise and reliable results. The obtained data aids healthcare professionals in diagnosing MTB complex infections, guiding treatment decisions, and monitoring patient response. Strict adherence to the provided instructions is imperative to ensure accurate interpretation of results and effective utilization of this diagnostic tool.

Kit Content

Ingredients	25 Preps (REF: ST242002)	100 Preps (REF: ST242006)
Pro MTB Mix	250 µl	1000 µl
QR-ROMAX, 4X	125 µl	500 µl
MTB Positive Control	100 µl	150 µl
RT-PCR Grade Water	100 µl	1500 µl

Storage

All components of the Stark MTB Molecular Diagnostic Kit are pre-prepared and ready for immediate use upon arrival. Upon receipt, it is recommended to store all reagents at temperatures ranging from -15°C to -30°C. These conditions ensure stability and maintain the integrity of the components until the expiration date indicated on the label.

Guarantee and Warranty

CARBON Technologies LLC stands behind the efficacy of all manufactured Kits and reagents. If you need assistance in choosing the right Kits for your needs, our technical support team is available to provide guidance. Should the products not meet your expectations due to reasons other than misuse, please do not hesitate to contact our technical support team. In the rare event of issues arising from the manufacturing process, CARBON Technologies LLC will promptly replace the Kit.

Warning and Precautions

- Material Safety Data Sheets (MSDS) for all products and reagents are available online at www.carbontechnologiesco.com. Users are advised to adhere to laboratory safety protocols diligently.
- Please review the guidelines thoroughly before initiating use.
- All patient samples and positive controls should be treated as potentially infectious.
- Consumption of food, beverages, tobacco, gum, cosmetics, or medication is strictly prohibited in

laboratories handling hazardous materials and human samples. All patient samples and positive controls must be regarded as potentially infectious.

- The Stark MTB Molecular Diagnostic Kit is intended for emergency and in vitro diagnostic use as directed by a physician's prescription.
- Each procedural step, including sampling, storage, shipping, and laboratory testing, must comply with biosafety and molecular laboratory management protocols.
- The Stark MTB Molecular Diagnostic Kit requires a dedicated and private laboratory space:
    - Location 1: Preparation Area - for test component preparation.
    - Location 2: Sample Processing Area - for isolation and control.
    - Location 3: Amplification Area - for Real-Time PCR testing.
- Clinical laboratories must be equipped with instruments and personnel compliant with Ministry of Health regulations.
- Altering or substituting any Kit components may impair its function and void the product license.
- All pipette tips and microtubes must be sterile and free from DNase and RNase contamination. To prevent cross-contamination, filter pipette tips should be used and replaced after each substance or sample addition.
- Dispose of waste in accordance with biosafety guidelines. Desks and laboratory instruments should be regularly disinfected with 70% ethanol or 10% sodium hypochlorite.
- Protect the Pro MTB combination from sunlight exposure.

Quality Control

The Stark MTB Molecular Diagnostic Kit undergoes rigorous testing in accordance with clinical, laboratory standards, and guidelines set forth by institutes such as the WHO. These tests are conducted through predefined experiments on a lot-to-lot basis to uphold consistent product quality. For your convenience, detailed results of all experiments can be accessed online by referencing the REF and Lot numbers at www.carbontechnologiesco.com.

Materials Required (but Not Provided)

- DNase-RNase-free microtubes (1.5 ml)
- PCR microtube 0.1 ml or 0.2 ml strip
- Various models of pipettes and pipette tips (10 µl, 100 µl, and 1000 µl of filter pipette tips)
- Surface sanitizing solution such as RNZO
- Disposable Powder-Free gloves and surgical gown
- Different types of Real-Time PCR Instruments (with green, yellow channels)
- Centrifuge (capable of reaching 13000 rpm)
- Microcentrifuge
- 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 transmitted from one person to another through tiny droplets released into the air via coughs and sneezes. Early detection of TB is paramount to improving health outcomes for individuals with TB and effectively reducing TB transmission. The Stark MTB Molecular Diagnostic Kit employs a polymerase chain reaction-based Real-Time PCR technique. Specifically designed for the qualitative diagnosis of the IS6110 gene (a specific multi-copy insertion sequence) of Mycobacterium tuberculosis, this Kit enables precise detection. After nucleic acid isolation using the DNJia Tissue and Bacteria Kit or other Ministry of

Health-approved Kits, the verified sample combination is added to the master mix primer/probe mix for the reaction. Furthermore, the Stark MTB Molecular Diagnostic Kit incorporates a secondary heterologous amplification system to identify potential PCR inhibition. This is detected as an internal control (IC) in the 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. Through its sampling mechanism, the quality of sample isolation and PCR reaction process can be monitored and controlled to prevent false-negative results. The assay demonstrates a Limit of Detection (LoD) of 25 Copies/ml.

Applications

The Stark MTB Molecular Diagnostic Kit is an in vitro nucleic acid amplification test designed 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, bronchoalveolar lavage (BAL), bronchial secretion, cerebrospinal fluid (CSF), stomach fluid, or peritoneal puncture samples. This diagnostic test Kit utilizes polymerase chain reaction (PCR) technology and is compatible with Real-Time PCR instruments.

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 initiating any tests, ensure that each component is thawed by gently bringing it to room temperature. Perform gentle up and down mixing, followed by a brief spin and centrifugation. Avoid subjecting the components to repeated freeze-thaw cycles.

Sample Storage and Preparation

For human sputum, bronchoalveolar lavage (BAL), bronchial secretion, cerebrospinal fluid (CSF), stomach fluid, or peritoneal puncture samples, the following guidelines apply:

Fresh specimens should either be processed immediately according to the sample procedure outlined in the Sample Processing Protocol section or stored frozen at -20°C. Prior to starting sample processing, frozen samples must be allowed to thaw to room temperature. Sample pre-treatment involves decontamination of the specimen, preparing it

for extraction.

Before Starting

Remove each component from the Kit and place them on the benchtop. Allow the reagents to equilibrate to room temperature. Then, gently mix each tube by performing up and down motions and briefly spin each tube in preparation for later use.

Buffer Preparation

Table1: Outlines the preparation of components per single reaction.

Components	Volume
QR-ROMAX, 4X	5µl
Pro MTB Mix	10µl
Isolated DNA	5µl

Pathogenicity

Mycobacterium tuberculosis is a species of pathogenic bacteria belonging to the family Mycobacteriaceae and is the causative agent of tuberculosis. It was first discovered in 1882 by Robert Koch. According to the most recent Global Tuberculosis Report (2019) edited by the World Health Organization (WHO), tuberculosis is ranked as the ninth leading cause of death worldwide and is the primary cause of mortality by a single infectious agent, with the highest rates of infection and mortality predominantly observed in developing and low-income countries.

Human infections with MTB typically begin through the inhalation of aerosol droplets containing tubercle bacilli directly expectorated from individuals with "open" pulmonary disease. The infectious dose for an individual is estimated to be between 1 and 200 bacilli; however, as a single aerosol droplet can contain anywhere from 1 to 400 bacilli, it remains uncertain what constitutes a biologically relevant dose. Following inhalation, the bacilli travel to the alveoli, where they are rapidly phagocytosed by alveolar macrophages.

The pathogenicity of MTB primarily stems from its ability to reprogram host macrophages after primary infection, thereby evading its own elimination. This includes the formation of granulomas, wherein the pathogen survives in equilibrium with host defenses, as well as the modulation of bacterial central metabolism and replication, leading to a dormant state wherein MTB becomes resistant to both host defenses and therapy.

Workstation Preparation

Before commencing work, ensure that all work surfaces, pipettes, centrifuges, and other supplies are thoroughly cleaned and sanitized. To minimize the risk of nucleic acid contamination, utilize sanitizers such as 70% Ethanol or 10% Sodium Hypochlorite.

Protocol

Thaw all reagents completely at room temperature (15–25°C). Once thawed, thoroughly mix all reagents by gently performing up and down motions, followed by brief spinning and centrifugation. Work promptly and maintain all reagents in the cooling block to preserve their integrity.

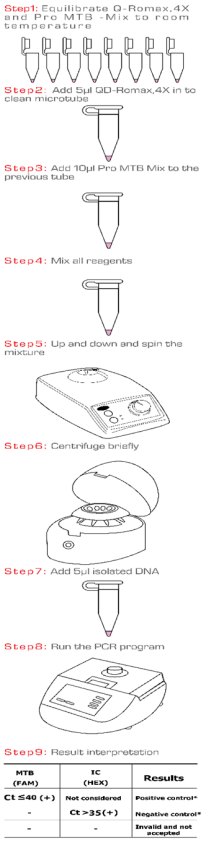

Figure 1: Preparation of reagents, PCR run, and interpretation of results

PCR Reaction Preparation

Table 2: PCR Reaction Preparation

Components	Volume
QR-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

- Perform data analysis for each gene separately using a manual threshold.
- Ensure that the threshold for each sample is set within the exponential phase of the fluorescence curves and above any background signal.
- Utilize the FAM Fluorophore (green) for the IS6110 gene of M. tuberculosis and the HEX Fluorophore

(Yellow) for the internal control (IC) gene.

- Employ a negative control to monitor contamination. Any significant increase in fluorescence curve magnitude in the negative control that does not cross the threshold, with a Ct value less than 35 (Ct<35), may indicate possible contamination. Strong signals above 35 in the negative control can suggest PCR artifacts; in such cases, consider the curve shape (an S-shaped curve typically indicates a positive result).
- Ensure that the internal control yields a positive result for all clinical specimens with a Ct value of 35 or less, indicating sufficient nucleic acid from the human gene and acceptable sample quality.
- A Ct value greater than 33 or an absence of Ct for the internal control indicates low sample concentration or inhibitors in the reaction. In such instances, it is recommended to dilute the isolated sample by at least half. If the test result remains unacceptable upon retesting, obtain a new sample from the patient and repeat the test.
- Positive clinical specimens should have a Ct value of ≤40 for the target gene (IS6110).
- If the expected positive reaction, characterized by a typical S-shaped curve, is not achieved, the performed test is deemed unacceptable and must be repeated in accordance with the Kit instructions.
- Identify the cause of the positive control failure, implement corrective actions, and document the results of corrective actions.

Acceptable Situations 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 may occur due to low titration of MTB in the patient sample, improper transportation, or inadequate sample isolation quality.
- Verification of all controls is essential before interpreting the results. If the controls are invalid, the patient's results cannot be accurately interpreted. The diagnostic threshold of this Kit is Ct≤40, and the user must review the fluorescence curve before making a final interpretation. All positive curves should exhibit an amplification peak.
- Failure to observe proper storage conditions for the Kit may lead to false-negative results.
- Handling of this Kit requires experienced and trained personnel. Any errors made by personnel may result in invalid results.
- The results obtained from this diagnostic Kit are considered acceptable only when supported by clinical evidence for diagnosing MTB. Definitive diagnosis and treatment decisions for patients should be based on a combination of this test with other test results, medical records, and the patient's response to treatment.

Performance Evaluation

- Preparation of a Standard Sample

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

- Limit of Detection (LoD) - Analytical Sensitivity

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

Analytical sensitivity considering the purification (DNJia Tissue and Bacteria Kit) of the Stark MTB Molecular Diagnostic Kit was determined using dilution series of plasmid standards from 50 to nominal 12.5 MTB Copies/ml spiked in clinical sputum specimens.

The LoD of each test was 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 Stark MTB Molecular Diagnostic Kit was established using the DNJia Tissue and Bacteria Kit. The results demonstrated that the LoD of the assay is 25 Copies/ml.

Detection Results of Stark MTB Molecular Diagnostic Kit Using DNJia Tissue and Bacteria Kit

Table 5: Determination of Stark 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

The inclusivity of the primer/probe set utilized in the Stark MTB Molecular Diagnostic Kit was assessed in silico using MTB sequences obtained from the NCBI database, accessed on September 26, 2021. The alignment analysis of the primer/probe sets for the IS6110 gene sequence revealed 100% inclusivity for MTB sequences identified from patient samples. Representative alignment results for the IS6110 gene are provided in the table below.

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 utilizing the DNJia Tissue and Bacteria Kit was conducted as supplementary data by testing three MTB-positive specimens. These specimens had been confirmed positive by the Stark 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. The results are summarized in the table below.

Table 7: Clinical sensitivity of Stark MTB Molecular Diagnostic Kit (CARBON Technologies)

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 analytical specificity of the Stark MTB Molecular Diagnostic Kit was assessed through both in silico analysis and

wet testing of 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 was conducted using the NCBI nr/nt database, accessed on September 28, 2021, utilizing the online BLASTN 2.10.0+ tool. Representative results of this analysis are provided in the table below. Furthermore, wet-testing confirmed the absence of cross-reactivity for other listed respiratory-borne pathogens, consistent with the in-silico findings.

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)

Clinical specificity of the nucleic acid of respiratory pathogens was assessed by testing the matrix of a negative sample (negative sputum) diluted with a certain concentration. Subsequently, the samples were extracted and tested using the Stark MTB molecular diagnostic Kit. No cross-reactivity was observed for other listed respiratory pathogens, as detailed in the table below.

Table 9: Investigation of the cross-reactivity of the MTB using Stark 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	-/-

- Accuracy Assessment

Intra-assay

Intra-assay precision assesses the ability of the method to determine the concentration of similar repeats within one Real-Time PCR run. Three repetitions of each concentration of the control sample were examined in one reaction, and coefficient of variation (CV) values were calculated for the threshold cycle (Ct) values. Results for the target gene (IS6110) showed a maximum coefficient of variation of 1.17 and a minimum coefficient of variation of 0.7. Acceptable results must have a CV of less than 5%.

Inter-assay

Inter-assay precision evaluates the results from different runs in Real-Time PCR or results from other laboratories. Five repeats of each concentration of the control sample were tested on three additional days. Results for the target gene (IS6110) showed a maximum coefficient of variation of 1.78 and a minimum coefficient of variation of 1.09. Acceptable results must have a CV of less than 10%.

- Clinical Evaluation

The clinical performance of the Stark MTB Molecular Diagnostic Kit was established using 130 human sputum, BAL, bronchial secretion, and CSF specimens collected from patients suspected of MTB. The comparator method was the Artus M. tuberculosis RG PCR Kit (Qiagen), which is CE-IVD certified. The extraction method used was the DNJia

Tissue and Bacteria Kit, and both assays were run on Rotor Q (Qiagen). The analysis demonstrated a Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) of 100%.
Clinical Comparison between Stark MTB Molecular Diagnostic Kit (CARBON Technologies) and Artus M. tuberculosis RG PCR Kit (Qiagen): The clinical assessment revealed a high level of agreement between the Stark MTB Molecular Diagnostic Kit and the Artus M. tuberculosis RG PCR Kit, with consistent performance in diagnosing MTB infections.

Table 10: Clinical Evaluation of Stark MTB molecular diagnostic Kit

Test		Artus M. tuberculosis RG PCR Kit (Qiagen)		Total
		Positive	Negative	
Stark 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%

Symbol


Manufacturer


Date of manufacture


Use-by date


Contains sufficient for <n> tests


Consult instructions for use


Biological risks


Temperature limit


CE Marking

EU Representative

In Vitro diagnostic medical device

Catalogue Number

Lot Number

Troubleshooting

For troubleshooting assistance with the Stark Genotyping HPV Molecular Diagnostic Kit, please consult the following guidelines. The CARBON Technologies LLC Technical Support Team is available to address any further questions or concerns you may have.

Problem	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

Technical assistance

For technical assistance, CARBON Technologies LLC ensures your complete satisfaction. Our technical support team comprises highly trained and experienced scientists adept at troubleshooting most problems you may encounter. They can offer expert advice to help you select the most suitable product for your needs.

You can contact our technical support team anytime through the following methods:

- Phone: +96897058350
- Directly submit your questions to the CARBON Technologies technical support team through our website: www.carbontechnologiesco.com
- Email your questions to: technicalsupport@carbontechnologiesco.com

Rest assured, our team is dedicated to providing prompt and effective assistance to address any inquiries or issues you may have. We are committed to ensuring your satisfaction and success in using our products.

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Release Date: ... ... Date of Manufacture: ... ...