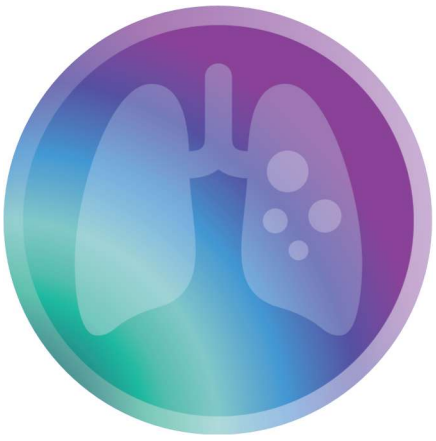
TB-Host Immune Response Assay

Research Use Only

Instructions for Use

English Version
Version issued: May 2025
Version: 1



Document reference number: Q1D-017
Product reference number: Q04-019-R01
For Research Use Only

1. Table of contents

2. Intended use

The QuantuMDx TB-Host Immune Response Assay (TB-HIRA) is a non-sputum, semi-quantitative real-time reverse transcription polymerase chain reaction (RT-qPCR) assay, based on a 6-gene transcriptomic signature (known as RISK6). RISK6 has been proposed as a tool for different aspects of tuberculosis (TB) management (Penn-Nicholson *et al.*, 2020, Bayaa *et al.*, 2021).

The RISK6 protocol comprises three steps: 1) cDNA preparation, 2) pre-amplification, and 3) singleplex RT-PCR of each transcript. The six transcripts are FCGR1B, GBP2, SDR39U1, SERPING1, TRMT2A, and TUBGCP6. A RISK6 score is calculated from the relative abundance of the six mRNA transcripts (either up or down regulated) in whole blood samples. QuantuMDx TB-HIRA simplifies the laboratory protocol for measuring the relative expression of these six transcripts by using a single, multiplexed 1-step RT-qPCR reaction yielding an analogous TB-HIRA score.

QuantuMDx TB-HIRA is intended for use by trained laboratorians who are proficient in performing molecular based tests.

QuantuMDx TB-HIRA is for 'Research Use Only'.

3. Principles of the procedure

The QuantuMDx TB-HIRA kit contains a 1-step multiplexed RT-qPCR assay to compare relative expression of six transcripts in RNA purified from human whole venous blood. The relative expression of these transcripts is used to calculate a score which can be used as a biomarker for TB.

Whole blood samples should be collected using appropriate third-party collection tubes (see section 6.1) and RNA purified using appropriate kits (see section 6.2). PCR should then be performed using the TB-HIRA 1-step RT-qPCR Multiplex Assay Master Mix included in the kit. The master mix contains all reagents required for the RT-qPCR amplification and quantification of the six human transcripts: SDR39U1, SERPING1, FCGR1B, TRMT2A, TUBGCP6 & GBP2. Once Cq values for each of the six transcripts have been determined, an algorithm is applied (see section 15) which compares the relative expression of the six transcripts and calculates the score for the sample.

4. Assay materials provided

The following materials are provided as part of the QuantuMDx TB-HIRA kit:

- Lyophilized TB-HIRA 1-step RT-qPCR Multiplex Assay Master Mix

There is sufficient reagent included within a single master mix vial of the QuantuMDx TB-HIRA Kit to run 100 reactions (reaction volume: 20 µl). Multiple batch runs can be performed from a single vial of the master mix.

5. Storage and handling conditions

The TB-HIRA kit is shipped at 15 - 25 °C. On arrival store away from light at 0 – 25 °C.

Rehydrated master mix should be stored at -20 °C. Repeated freeze/thaw cycles should be avoided and not exceed five times. Solutions should be mixed after each thawing.

Do not use reagents after their labelled expiration date.

6. Materials and equipment required but not provided

- Sample collection tubes*
- RNA extraction/purification kit**
- DNase (if not included as part of RNA extraction/purification kit) †
- Thermocycler with 6-channel detection††
- Positive Control (PC)‡
- PCR grade water
- PCR tubes or plates
- Plate sealer and optically transparent film
- Benchtop microcentrifuge
- Vortex mixer
- Adjustable micropipettes
- Racks for microcentrifuge tubes
- 1.5 mL microcentrifuge tubes (DNase/RNase free)
- Aerosol barrier pipette tips, various sizes
- Ice
- Plate spinner
- Biological safety cabinet
- -20 °C ± 10 °C Freezer
- -80 °C ± 10 °C Freezer
- 2 to 8 °C Refrigerator
- Biohazard bag for tips and tube disposal
- Cleaning and decontamination reagents such as 0.5% sodium hypochlorite, 70% ethanol, DNAzap™ or RNase AWAY®
- Disposable gloves
- Eye protection
- Lab coat

* See section 6.1 for more information
** See section 6.2 for more information
† See section 6.3 for more information
†† See section 6.4 for more information
‡ See section 6.5 for more information

6.1. Specimen collection tubes

QuantuMDx TB-HIRA has been developed using blood collected using the tube type listed in Table 1. Other collection tubes may be suitable but will require validation by the user. Use of non-validated sample collection tubes may result in incorrect results.

Table 1 Specimen collection tubes validated for use with QuantuMDx TB-HIRA

Manufacturer	Product name	Product code	Volume whole blood required
Qiagen	PAXgene® Blood RNA tubes	762165	2.5 ml
Applied Biosystems™	Tempus™ Blood RNA Tube	4342792	3 ml

6.2. RNA extraction Kits

QuantuMDx TB-HIRA has been developed using the nucleic acid extraction kit listed in Table 2. Other kits may be suitable for use but will require validation by the user. Use of non-validated kits may result in incorrect results.

Table 2 RNA extraction kits validated for use with QuantuMDx TB-HIRA

Manufacturer	Kit	Sample tube type*	Product code	DNAse treatment included
Qiagen	PAXgene® blood RNA kit	PAXgene® blood RNA tubes	762174	Yes
Applied Biosystems™	Tempus™ spin RNA isolation kit	Tempus™ blood RNA tube	4380204	No

* RNA extraction kits used should be appropriate for the sample collection tube type. For more information on sample types refer to sections 6.1 and 9.

6.3. DNase treatment kits

If RNA is extracted using a kit which does not include a DNase treatment step, this should be performed as an additional step. Validated DNase kits are listed in Table 3. Whether sample extraction kits include DNAse treatment is indicated in Table 2. Other kits may be suitable for use but will require validation by the user. Use of non-validated kits may result in incorrect results.

Table 3 DNase kits validated for use QuantuMDx TB-HIRA

Manufacturer	Kit	Product code
Invitrogen	TURBO DNA-free™ Kit	AM1907

6.4. Real-time thermocycler instruments

QuantuMDx TB-HIRA has been developed using the real-time thermocycling instrument and associated controller software listed in Table 4. Other six channel qPCR thermocyclers may be suitable but will require validation by the user.

Table 4 Thermocycler instruments validated for use with QuantuMDx TB-HIRA

Manufacturer	Model	Controller software (version)
Applied Biosystems™	QuantStudio™ 5	QuantStudio Design and Analysis software (1.5.2)

All instruments should be calibrated for the fluorophores FAM, HEX, TAMRA, ROX, Cy5 and Cy5.5 and maintained according to the manufacturer's instructions and recommendations.

6.5. Controls

This kit does not include a positive control (PC) or negative control (NC).

The NC should be PCR grade water.

The materials shown in Table 5 have been demonstrated as suitable for use as a PC.

Table 5 Material validated for use as a positive control for QuantuMDx TB-HIRA

Manufacturer	Product name	Product code
Thermo Fisher	Total RNA Control (Human)	4307281

7. Facility and training requirements

Testing for TB-HIRA should be performed in an appropriately equipped and maintained laboratory. Staff should be trained in the relevant technical and safety procedures.

8. Precautions and handling requirements

8.1. Warnings and Precautions

- This kit is intended for research use only. Results should not be used for clinical diagnosis.
- Blood samples may contain pathogens other than *M. tuberculosis*. All samples should be considered biohazardous and handled using universal precaution. Only personnel proficient in handling infectious materials should perform this procedure.
- Good laboratory practices are essential to ensure proper performance of the assay. Care should be taken to ensure there is no contamination of reagents.
- Personal protective equipment (PPE) including lab coats, gloves, eye protection and a biological safety cabinet are recommended for manipulation of specimens. All specimen processing should be performed in accordance with national, local and institutional biological safety recommendations.
- Wash hands thoroughly after handling specimens and kit reagents as well as after removing gloves.
- If spillage of specimen should occur, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite (5,000 mg/L available chlorine), or follow appropriate procedures as outlined by the site or facility.
- In case of contact with potentially biohazardous material, consult local risk assessment.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents, specimens and samples are handled.
- Decontaminate and dispose of all potentially infectious materials in accordance with relevant institutional, local and national regulations.
- Dispose of all cleaning materials as biological waste.
- Safety Data Sheets (SDS) are available upon request and on the QuantuMDx website at www.quantumdx.com/Support-Documentation/.
- Closely follow procedures and guidelines provided. Any deviation from the procedure and guidelines may affect assay performance.

8.2. Reagent handling

- When handling reagents, always use appropriate PPE including lab coats, gloves and eye protection.
- Do not use assay components beyond their recommended storage or expiry dates.
- Before use, visually inspect all reagents to ensure that there are no signs of breakage or leakage. If there are signs of breakage or leakage, DO NOT use the material for testing and immediately contact QuantuMDx.
- If spillage of reagent should occur, wipe down surfaces with a damp cloth in accordance with the procedure outlined in the SDS.
- Should the reagent vial become damaged, clean up and dispose of broken glass in accordance with local procedures.

- Handle all reagents, controls, and specimens according to good laboratory practice. This is essential to minimize the risk of cross-contamination between samples or the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with nucleic acids.
- Avoid microbial and nuclease contamination of the PCR reagent when removing aliquots. Use sterile, disposable, nuclease-free, aerosol barrier pipette tips.
- Change pipette tips between all liquid transfers. Tips are discarded after use.
- Work area and instrument platforms must be considered potential sources of contamination. Change gloves after contact with potential contaminants (specimens, eluates, and/or amplified product) before handling unopened reagents, controls, calibrators, or specimens.
- Extracted RNA should be maintained on ice during preparation and use. RT-qPCR reagent and mixtures should be maintained on ice during preparation.
- Add reagents to the bottom of the reaction tube or reaction well without touching the pipette tip to the rim or side of the tube or well.
- Set up each run separately.
- Dispose of unused kit reagents and human specimens according to all relevant institutional, local and national regulations.

8.3. Prevention of nucleic acid contamination

Amplification technologies, such as PCR, are sensitive to accidental introduction of product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the PCR reagents used in the amplification step become contaminated by accidental introduction of amplification product. Measures to reduce the risk of contamination in the laboratory include physically separating the activities involved in performing PCR in compliance with good laboratory practices and establishing a unidirectional workflow. The possibility of nucleic acid contamination is minimized when:

- PCR reactions are prepared in a dedicated PCR workstation away from sources of contamination.
- Oligonucleotide hybridization and PCR amplification occur in a properly sealed plate or reaction tube.
- Detection is carried out automatically without the need to open the reaction vessel.
- Once the reaction has been completed, the reaction plate or tubes should never be opened and should be disposed of properly.
- Unidirectional workflow practices are followed, such that reaction well aliquoted PCR reagent is brought to the target preparation location for mixing, then the complete reaction plate to the instrument location.
- Work surfaces and equipment are regularly cleaned with the appropriate solutions (see section 6).

9. Sample collection, handling and storage

The validated sample type for this assay is venous blood collected in tubes intended for the stabilisation of RNA.

9.1. Specimen Collection

Refer to the specimen collection tube manufacturer's package insert or facility's procedures. See section 6.1 for specimen collection tubes demonstrated as suitable for use with QuantuMDx TB-HIRA.

9.2. Specimen Storage

Refer to specimen collection tube manufacturer's package insert for guidance on storage of unprocessed samples. Purified nucleic acids should be aliquoted and stored at -80 °C ± 10 °C.

Caution: The following can affect the results obtained:

- Inadequate or inappropriate collection of specimens
- Incorrectly stored specimens
- Incorrectly transported specimens
- Use of non-validated specimen matrices
- Inadequate or excessive specimen volume

10. RNA purification

RNA should be purified from whole blood using off-the-shelf kits appropriate for the specimen collection tube type used. Kits recommended for use with QuantuMDx TB-HIRA are listed in Table 2. RNA purification should be performed in accordance with the relevant manufacturer's instructions for use.

After purification, RNA should be maintained on ice or aliquoted and stored at –80 °C if not immediately required.

Caution: RNA must be DNase-treated prior to performing RT-qPCR. Depending on the RNA extraction kit used, this may be an optional or mandatory step; in cases where this is an optional step this must be performed. The PAXgene blood RNA kit includes DNase treatment as a mandatory step therefore no further action is required.

Caution: Samples collected using PAXgene Blood RNA Tubes must be incubated at room temperature (15 to 25 °C) for a minimum of 2 hours before purification to achieve complete lysis of blood cells and precipitation of RNA. If the initial blood incubation at room temperature for was not done before refrigeration or freezing, equilibrate the PAXgene Blood RNA Tube to room temperature and then incubate for 2 hours before processing.

11. Controls

Controls are not included as part of this kit and must be provided by the user. See section 6.5 for more information on recommended control material.

Quality controls should be performed in conformance with local and national regulations, accreditation requirements, and the standard quality control procedures of your laboratory.

The PC should be considered a pass if amplification is observed in all channels.

The NC should be considered a pass if no amplification is observed on any channel or if amplification is observed on a single channel with a Cq greater than 37.

A PC should be tested prior to running samples with each new kit lot to ensure proper functioning of all reagents, kit components and real-time thermocycler.

NC and PC should be included in every batch run performed. The number of NC and PC performed per batch run should match the number of technical replicates performed per sample.

Failure of the controls (PC or NC) invalidates the run. Results should not be reported and repeat testing should be done starting from purified nucleic acid, using a new aliquot of PC. The function of PC and NCs are summarised in Table 6.

Table 6 Function of controls

Control Type	Used to monitor
Positive (PC)	• Substantial reverse transcriptase or polymerase failure • Substantial primer and probe failure • Ensure correct thermocycler settings (program & calibration)
Negative (NC)	• Reagent contamination • Environmental contamination

In the case of higher-than-expected numbers of test failures and/or suspected inaccurate test results, please contact QuantuMDx.

12. Rehydration of lyophilised Master Mix

1. Ensure all dried material is collected at the bottom of the vial. If necessary, with the vial held upright gently flick the top of the vial until the powder is settled at the bottom.
2. Open the vial and add 1550 µl PCR grade water.
3. Mix by pipetting up and down 10 times.
4. After rehydration, master mix should be kept on ice and protected from light, or stored at -20 °C.

13. Preparation of RT-qPCR reactions

Caution: Clean and decontaminate all work surfaces, pipettes, centrifuges, and other equipment prior to use. Decontamination agents should be used such as 0.5% sodium hypochlorite, 70% ethanol, DNAzap™ or RNase AWAY® to minimize the risk of nucleic acid or RNase contamination.

Caution: All reagents and purified RNA should be maintained on ice during preparation of the reaction and until ready for PCR.

Caution: Fluorescent probes used are photosensitive. TB-HIRA PCR Master Mix should be stored away from light and tubes/plate should be covered with aluminium foil if left on ice for prolonged periods of time (>30 min).

Caution: Repeated freeze/thaw cycles of TB-HIRA PCR Master Mix should be avoided and not exceed five cycles.

1. For first use, rehydrate Master Mix as described in Section 12. If Master Mix was previously frozen, thaw on ice. Briefly vortex to ensure reagent is well mixed and ensure reagent is collected at the bottom of the tube by briefly centrifuging or gently tapping the tube.
2. Using an appropriate methodology, measure the concentration of RNA in the extracted sample(s).
3. Using the volumes described in Table 7, prepare reaction mixtures in a PCR plate or tubes. At least one PC and one NC should be included per each reaction batch.

Table 7 Component volumes for PCR reaction mix

Component	Volume (µl)
Water	X*
Sample	Y*
Master Mix	15
Final Volume	20

*X + Y = 5 µl. Mixture should contain at least 25 ng RNA. Add an appropriate volume of sample to contain at least 25 ng RNA and make up to 5 µl with water.

4. Seal plate or tubes. Ensure wells or tubes are completely sealed to avoid evaporation of samples during PCR.
5. Briefly spin the plate or tubes to ensure mixture is collected at bottom of wells.
6. Transfer plate or tubes to PCR thermocycler and begin PCR as soon as possible.

14. Thermocycling conditions

PCR should be performed using the thermocycling conditions shown in Table 8. Data collection is made through the FAM, HEX, ROX, TAMRA, Cy5 and Cy5.5 channels during the annealing/extension step.

Table 8 Thermocycler run parameters

Step	Temperature (°C)	Time (mm:ss)	Cycles
Reverse transcription	55	10:00	1
Polymerase activation	95	2:00	1
Denaturation	95	0:05	40
Annealing / Extension	63	0:20*	

*Minimum 20 s, time may be extended if necessary to facilitate data collection.

To ensure best performance of the assay on any given thermocycler instrument, additional changes to settings may be required. Settings for recommended thermocycler instruments are given below. In each case all other settings should be left at their default as per the manufacturer's instructions for use.

14.1. Applied Biosystems™ QuantStudio™ 5

Before first use of the TB-HIRA assay, the instrument should be set to collect data using all appropriate excitation & emission filter combinations and the instrument must be calibrated to detect Cy5.5. Dye calibration should be performed in accordance with the manufacturer's instructions for use. When performing this calibration, from the three--dot menu on the Run Method tab, select Filter Settings. The following filter combinations should be selected: x1-m1, x1-m2, x2-m1, x2-m2, x2-m3, x3-m2, x3-m3, x3-m4, x4-m3, x4-m4, x4-m5, x5-m4, x5-m5, x5-m6, m6-x5, x6-m6.

When performing the assay, filter settings should match those used during dye calibration.

When performing primary analysis to determine Cq values from raw data, the Relative Threshold method should be used.

15. Calculation of TB-HIRA score

Information: Calculation of TB-HIRA scores use a set of score tables to compare relative expression of gene pairs and assign significance. Score tables currently used are identical to those developed by Penn-Nicholson *et al.* (2020) for calculation of RISK6 scores from six, single-plex RT-qPCR reactions. Due to the multiplexed nature of the QuantuMDx TB-HIRA assay, Cq values and subsequently scores calculated may not be identical to RISK6 scores. Score tables optimised for use with the multiplexed TB-HIRA assay are in development and will be made available once validated.

Caution: All assay controls must be examined prior to interpretation of test sample results. The NC should be considered a pass if no amplification is observed on any channel or if amplification is observed on a single channel with a Cq greater than 37. The PC should amplify all transcripts. If the controls are not valid and acceptable, the run is invalid and must be repeated. If repeated control failures are observed, consult QuantuMDx for assistance.

Caution: Amplification plots should be examined for every sample. If the amplification plot shows an exponential increase, the amplification curve is valid. All samples should be analysed for the presence of baseline drift if corrections are not applied via the instrument controller software. In instances where background fluorescence appears high, the initial PCR cycles can be omitted from analysis.

To calculate the TB-HIRA score, first determine Cq values for each of the six transcripts. See Table 9 for a list of transcripts and their corresponding fluorophores.

Table 9 Transcripts and their corresponding fluorophores

Transcript	Fluorophore
TRMT2A	FAM (6-Carboxyfluorescein)
TUBGCP6	HEX (Hexachlorofluorescein)
SDR39U1	TAMRA (Tetramethylrhodamine)
SERPING1	ROX (Carboxy-X-Rhodamine)
GBP2	Cy5 (Cyanine5)
FCGR1B	Cy5.5 (Cyanine5.5)

Scores are calculated using the algorithm developed by Penn-Nicholson *et al.* (2020). Tools have been built in excel to calculate TB-HIRA scores from Cq values, either by

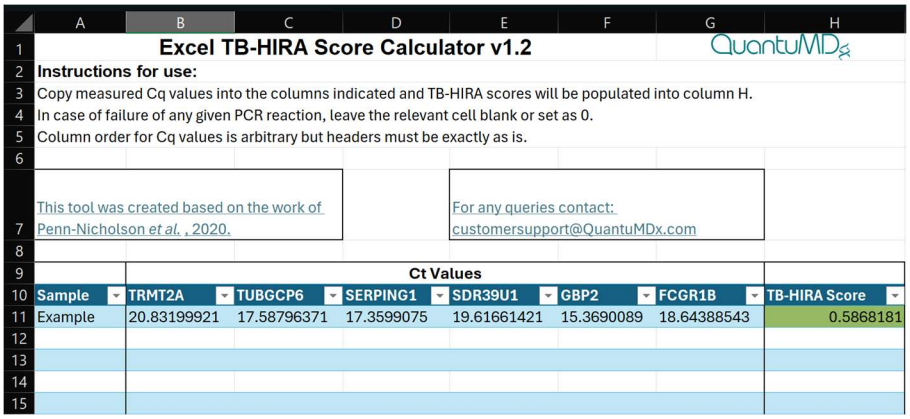
manually inputting Cq values or automatically parsing values exported from Thermocycler control software. Tools are available to download from:

https://www.quantumdx.com/support-documentation/

15.1. TB-HIRA Score Calculator 1.x

The TB-HIRA Score Calculator 1.x is built to allow free entry of Cq values and will automatically return a TB-HIRA Score. To use the TB-HIRA 1.x Score Calculator:

1. Download the TB-HIRA Score Calculator 1.x from the QuantuMDx website and open in Excel.
2. Save the file locally and enable editing.
3. (Optional) enter sample names into the sample name column.
4. Enter Cq values for each of the six transcripts into the relevant column. The TB-HIRA score will automatically be calculated and returned.



Excel TB-HIRA Score Calculator v1.2

Instructions for use:

Copy measured Cq values into the columns indicated and TB-HIRA scores will be populated into column H.
In case of failure of any given PCR reaction, leave the relevant cell blank or set as 0.
Column order for Cq values is arbitrary but headers must be exactly as is.

This tool was created based on the work of Penn-Nicholson *et al.* , 2020.

For any queries contact: customersupport@QuantuMDx.com

	Ct Values						
Sample	TRMT2A	TUBGCP6	SERPING1	SDR39U1	GBP2	FCGR1B	TB-HIRA Score
Example	20.83199921	17.58796371	17.3599075	19.61661421	15.3690089	18.64388543	0.5868181

Figure 1 Screengrab showing the Excel TB-HIRA Score Calculator 1.x

15.2. TB-HIRA Score Calculator 2.x

The TB-HIRA Score Calculator 2.x parses Cq values from data exported from qPCR analysis software. This tool was developed to parse data exported from Applied Biosystems™ Design and Analysis 2 (DA2), but can also parse data exported from other software packages. To use the TB-HIRA 2.x Score Calculator:

1. Download the TB-HIRA Score Calculator 2.x from the QuantuMDx website and open in Excel.
2. Save the file locally and enable editing.
3. In qPCR primary analysis software, ensure data is fully annotated including sample names and gene targets. NB gene targets must be given as TRMT2A, TUBGCP6, SERPING1, SDR39U1, GBP2 and FCGR1B exactly.

4. Export Cq values from primary analysis software. When using DA2 export data with default settings.
5. Open the data export file. When using DA2, to analyse individual wells open the exported file including _Analysed_Results_ in the name; to analyse results as replicate groups open the file including _Analysed_Replicate Group Result_ in the name.
6. Select a free cell in the export file and copy the whole sheet using Ctrl + A, Ctrl + C.
7. Open the TB-HIRA Score Calculator 2.x and select the Input tab.
8. Select cell A1 and past the exported data using Ctrl + V.
9. Navigate to the output tab. From the dropdown menu select the input sheet type. Some different versions of DA2 use different formatting and therefore the correct version must be selected. If using primary analysis software other than DA2, the sheet can parse data if data is presented as a table including columns for Sample Name, Target Names and Cq values. Select Other as the Input sheet type, update all boxes to indicate the columns containing required data and the first row containing data. The sheet will then automatically parse data and calculate the TB-HIRA Score.

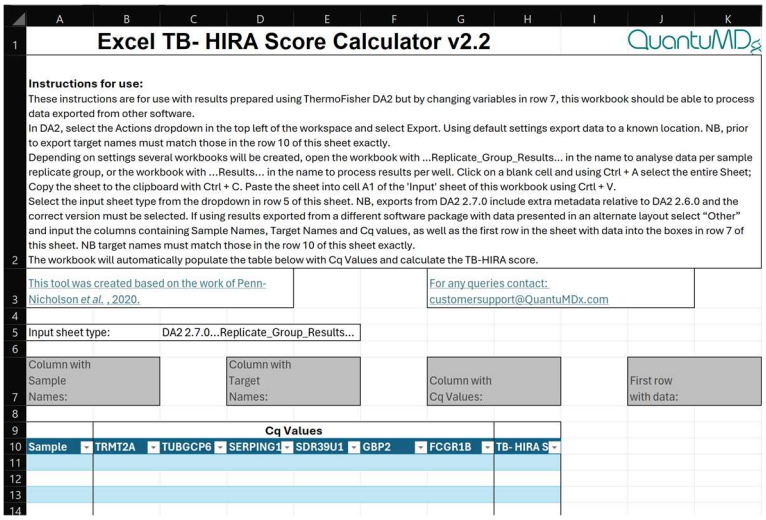


Figure 2 Screengrab showing the Excel TB-HIRA Score Calculator 2.x

15.3.R

Alternatively, TB-HIRA scores can be calculated in R using the protocol of Penn-Nicholson *et al.* (2020). The R script used in this paper can be downloaded from:

https://bitbucket.org/satvi/risk6/.

16. Interpretation of results

Caution: The QuantuMDx TB-HIRA kit is currently for Research Use Only. Results should not be used for clinical diagnosis.

Caution: RISK6 scores are determined from six single-plex RT-qPCR reactions, whereas QuantuMDx TB-HIRA uses a single multiplex RT-qPCR reaction. As a result, scores calculated may not match scores determined in accordance with Penn-Nicholson *et al.* (2020) and should be interpreted accordingly.

Information: The QuantuMDx TB-HIRA kit is undergoing ongoing development and guidance on how scores should be interpreted is under development.

17. Limitations

Training and familiarity with testing procedures and interpretation of results is necessary prior to performing the assay.

Performance of QuantuMDx TB-HIRA has only been established in RNA purified from whole blood specimens. Other specimen types may yield inaccurate results.

The detection of nucleic acid is dependent upon proper specimen collection, handling, transportation, storage, and preparation, including extraction. Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of incorrect values resulting from improperly collected, transported, or handled specimens.

This assay cannot rule out infections caused by other pathogens.

The prevalence of TB will affect the test's predictive value.

Once reactions are established in tubes/plates, thermal cycling should proceed immediately.

Samples may contain pathogens not limited to *M. tuberculosis*.

18. Performance characteristics

Internal testing has demonstrated the assay was able to reproducibly detect and quantify the six target transcripts across all expected reaction conditions.

Testing was performed using different RNA preparation protocols (PAXgene® tubes and extraction kit; Tempus™ tubes and extraction kit; Thermo Fisher control RNA), RNA concentrations (5, 25 & 125 ng/rxn) and immune response profiles (interferon stimulated, non-interferon stimulated). In all conditions tested, repeatability was determined for each of the six transcripts with respect to different reagent vials, operators, qPCR machines and on different days; in all cases the coefficient of variance for each transcript was < 5%.

Resistance of reconstituted master mix to up to 5 freeze thaw cycles was confirmed. Rehydrated TB-HIRA master mix was exposed to 1, 3 or 5 freeze thaw cycles and no significant change in performance was observed.

19. Inclusivity

TB-HIRA targets the same transcripts as detected in the RISK6 protocol from which it is derived (Penn-Nicholson *et al.*, 2020). *In silico* analysis was performed comparing sequences of primers and probes with target sequences from RefSeq and GenBank. It is expected that all target sequences should be detected.

20. Exclusivity

The amplicons generated from the primers and probes for the TB-HIRA Lab Assay were analysed *in silico* for cross-reactivity with microorganisms potentially found in blood samples. Based on publicly available sequences in the NCBI core database (as of November 2024), the six amplicons generated in the TB-HIRA lab assay do not show more than 80% identity with any of the microorganisms tested (Table 10).

Table 10 Organisms tested for potential cross-reactive with TB-HIRA

Organism	Taxonomy identifier
Human immunodeficiency virus (HIV)	12721
Entamoeba histolytica (Amoeba)	5774
Arbovirus Group A (*Alphavirus*, *Togaviridae*)	11019
Arbovirus Group B (*Flaviviridae*)	11051
Aspergillus	5052

Babesia (Babesiosis)	5864
Bordetella	517
Borrelia burgdorferi	139
Brucella (*B. abortus, B. canis, B. meliensis* and *B. suis*)	234
Candida/ *Lodderomyces* (*albicans*, *auris*, *dubliniensis*, *matapsilosis*, *parapsilosis*, *tropicalis*)	1535325
Chikungunya virus (*Alphavirus*)	37124
Clavispora lusitaniae (previously *Candida lusitaniae*)	36911
Coxiella burnetii (Q fever)	777
Cryptococcus (*neoformans* or *gattii*)	5206
Human cytomegalovirus	10359
Dengue fever virus (*Flavivirus*)	12637
Corynebacterium diphteria	1717
Ebola virus	1570291
Epstein Barr virus	10376
Escherichia coli	562
Filaria (roundworm)	221949
Haemophilus	724
Hantavirus	1980442
Human hepatitis virus A	208726
Hepatitis B virus	10407
Human hepatitis virus C	11103
Hepatitis delta virus (D)	12475
Human hepatitis virus E	291484
Hepatitis virus G virus isolate PEI	140024
Herpes Simplex virus 1	10298
Herpes Simplex virus 2	10310
Human T-cell leukaemia virus type I	11908
HTLV 2 (Human T-lymphotropic virus)	11909
Echinococcus granulosus (flatworm)	6210
Kluveromyces marxianus (previously *Candida kefyr*)	4911
Lassa fever virus (*Mammarenavirus lassaense*)	3052310
Leishmania (protozoa)	5658
Leptospira (*interrogans*, *kirschneri*...)	171
Plasmodium falciparum (Malaria)	5833
Marburg virus (*Orthomarburgvirus*)	3044783
Measles (*Morbillivirus*)	11234

Neisseria meningitidis (Meningococcal disease)	487
Mycobacterium tuberculosis	1773
Nakaseomyces glabratus (previously *Candida glabrata*)	5478
Pseudomonas aeruginosa	287
Parvovirus (*Parvoviridae*)	10780
Pichia kudriavzevii (previously *Candida krusei*)	4909
Rickettsia (*Rickettsiales*)	766
Rubella virus	11041
SARS-CoV-2	2697049
Schistosoma	6181
Staphylococcus aureus (MRSA)	1280
Streptococcus pneumoniae	1313
Streptococcus pyogenes	1314
Roundworms (Nematodes)	6231
Clostridium tetani	1513
Toxocara	6264
Toxoplasma	5810
Treponema pallidum (syphilis)	160
Varicella-Zoster virus (chicken pox)	10335
West Nile Virus	11082
Zika virus	64320

21. Interfering substances

The input sample for the TB-HIRA assay is purified RNA. For information on applicable interfering substances tested for your chosen RNA extraction/purification kit, please refer to the relevant IFU. Examples of interfering substances which could be present in blood samples are:

- Human haemoglobin
- Triglycerides
- Unconjugated bilirubin
- Anticoagulants (e.g. K_2EDTA, K_3EDTA, Na_2EDTA)
- Alcohol
- Various drugs

22. Disposal

Dispose of hazardous or biologically contaminated materials in accordance with local laws and according to the practices of your institution.

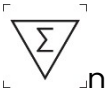
23. References

Bayaa, R., Ndiaye, M.D.B., Chedid, C. *et al.* Multi-country evaluation of RISK6, a 6-gene blood transcriptomic signature, for tuberculosis diagnosis and treatment monitoring. *Sci Rep* **11**, 13646 (2021). https://doi.org/10.1038/s41598-021-93059-1

Penn-Nicholson, A., Mbandi, S.K., Thompson, E. *et al.* RISK6, a 6-gene transcriptomic signature of TB disease risk, diagnosis and treatment response. *Sci Rep* **10**, 8629 (2020). https://doi.org/10.1038/s41598-020-65043-8

24. Symbols in use

Table 11 Symbols in use

Symbol	Interpretation	Symbol	Interpretation
	Consult instructions		Indicates the kit contents sufficient for <n> tests
	Indicates the product's temperature limits		Indicates Use by Date
	Indicates the product batch code		Indicates the product's reference number
	Indicates the name and location of the product manufacturer		Fragile
	Keep away from sunlight		Indicates the product's humidity limits

25. Revision history

An indication of the introduced modifications for each revision will be listed below.

Table 12 Revision history

Revision	Modifications
1	• Document introduced

26. Contact information

For customer support please contact QuantuMDx directly. Information can be found at:

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Telephone hours:	09:00 – 17:00 (GMT/BST)

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