A near-patient, non-sputum based, multiplex RT-PCR assay for tuberculosis diagnosis and disease monitoring

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INTRODUCTION

Accurate diagnosis of tuberculosis (TB) infection remains a major global health challenge. Worldwide in 2021:[1]

- 10.6 million people became ill with TB, including 1.2 million children
- 60% cases (6.4 million) were detected and notified
- 1.5 million people died from TB

Host-response blood-based diagnostics are an area of diagnostic interest.

RISK6 is one such PCR-based transcriptomic signature that is being investigated for diagnosis of tuberculosis, prediction of disease risk, and monitoring of treatment response, from whole blood samples.[2]

Currently, the six transcripts are detected using singleplex qPCR ThermoFisher TaqMan™ gene expression assays, of which the primer and probe sequences are not available in the public domain.

Objective

To develop a multiplex one-step RT-qPCR assay that simultaneously amplifies and detects RISK6 transcripts directly from extracted RNA in a

1. single reaction using a benchtop thermal cycler (Fig.1)
2. microfluidic shuttle-flow PCR system (sf-PCR)

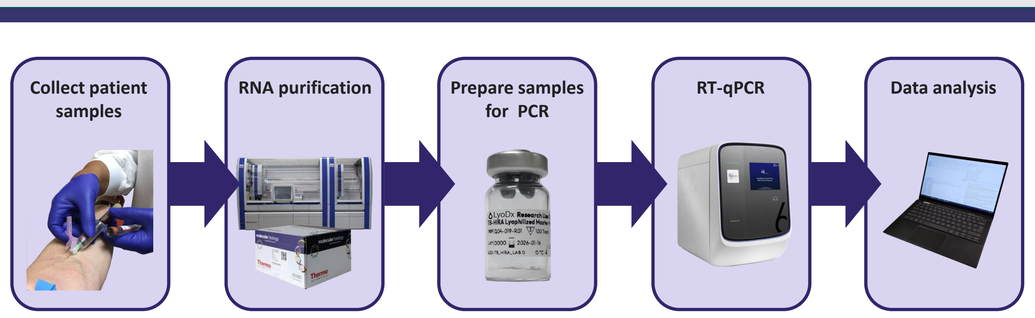

Figure 1: Proposed workflow for the multiplex one-step RT-qPCR assay.

RISK6 EXPLAINED

RISK6

- determines the relative abundance of one mRNA transcript to a partner transcript
- comprises nine pairs linking a transcript that is upregulated (red boxes) during TB progression with one that is downregulated (green boxes) relative to healthy controls (Fig. 2)

RISK6 scores are calculated based on delta Cts for the nine transcript pairs.[2]

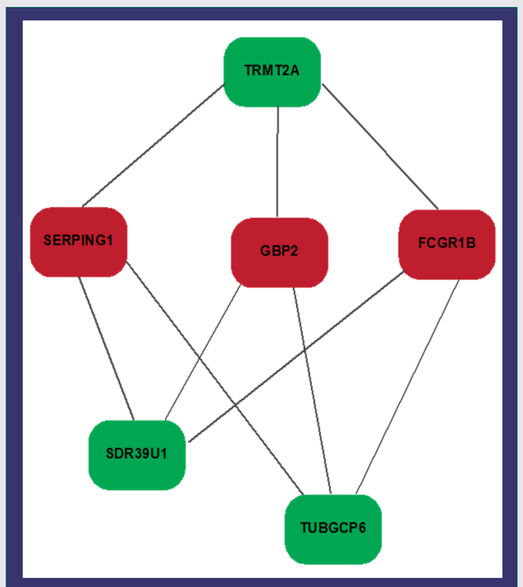

Figure 2: Interlinking (black lines) relationship of RISK6 transcripts.

METHODS

Alternative primers and probes to the six transcripts were designed.

The best performing primers were selected and singleplex Taqman™ assays were developed. Each singleplex assay was compared with and validated against a commercially available assay (Thermo Fisher Scientific).

The one-step singleplex RT-qPCR assays were combined and a six channel, multiplexed assay was developed.

The multiplexed RT-qPCR was ported to sf-PCR to demonstrate compatibility with the QuantuMDx Q-POC™ point-of-care PCR instrument.

Performance of the multiplexed assays was verified using 30 RNA samples extracted from venous blood provided by SATVI* :

- 15 from individuals with TB infection (controls)
- 15 samples from individuals with active TB (cases)

Risk scores generated by the original RISK6 protocol (as used by SATVI), the multiplex one-step RT-qPCR assay on the benchtop thermocycler and on the sf-PCR were compared.

ACKNOWLEDGEMENTS

Our thanks go to Professor Tom Scriba and colleagues at the *South African Tuberculosis Vaccine Initiative (SATVI) and to the individuals who donated blood samples.

RESULTS

- RNA samples from individuals with active TB had a higher risk score (>80%) than controls (<80%) (Fig. 3a).
- Risk scores generated by the one-step multiplex RT-qPCR assay on the benchtop thermocycler and on the sf-PCR system were comparable to the scores generated by the original RISK6 protocol for TB cases (Fig. 3a).
- Positive correlation was observed between the risk scores generated by the one-step multiplex RT-qPCR assay on the benchtop thermocycler and on the sf-PCR system with the risk scores generated using the original RISK6 protocol (Fig. 3b & c).

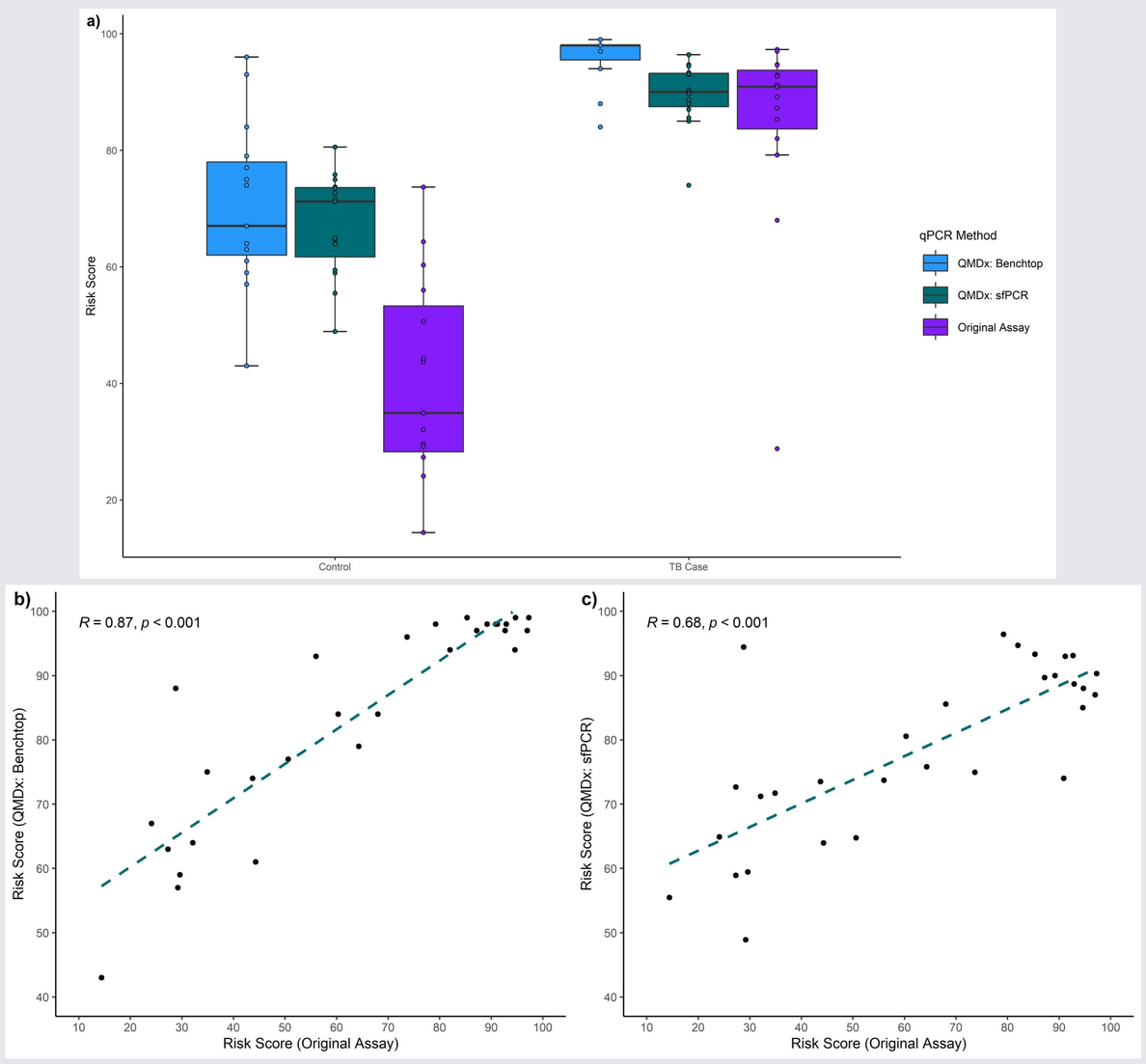

Figure 3: Comparison of risk scores generated by the multiplex one-step RT-qPCR assay on a benchtop thermocycler and on the sf-PCR system with scores generated by the original RISK6 protocol from RNA samples extracted from venous blood [a]. Correlation between the one-step multiplex RT-qPCR assay on the benchtop thermocycler [b] and on a sf-PCR system [c] with the risk scores generated using the original RISK6 protocol.

CONCLUSIONS

We developed a multiplex one-step RT-qPCR assay that simultaneously amplifies and detects six mRNA transcripts directly from extracted RNA in a single reaction on a benchtop thermocycler and on a sf-PCR system.

The laboratory-based TB-Host Immune Response Assay (TB-HIRA) is:

- based upon a published 6-gene transcriptomic signature (RISK6)
- a non-sputum-based assay
- currently for Research & Development Use Only

Potential clinical utility in tuberculosis diagnosis and management:

- Diagnosis of active pulmonary tuberculosis
- Monitoring response to TB treatment without the need for *Mycobacterium tuberculosis* culture

FUTURE DEVELOPMENTS

We are developing the laboratory-based TB-HIRA for use:

- to automatically generate a risk score
- in ~1 hour
- with a small volume of whole blood (µL) from a dried blood spot

REFERENCES

[1]WHO. Tuberculosis Factsheet. 21 Apr 2023. https://www.who.int/news-room/fact-sheets/detail/tuberculosis

[2]Penn-Nicholson, A., *et al.* 2020. RISK6, a 6-gene transcriptomic signature of TB disease risk, diagnosis and treatment response. *Sci Rep.* May 25;10(1):8629. doi: 10.1038/s41598-020-65043-8