Sample collection procedures followed in Phase I studies

Whole blood (PAXgene RNA)

PAXgene Blood RNA tubes were utilized for collecting and stabilizing blood RNA. These tubes are kept upright at all times at room temperature (17-25°C) before use and during blood collection. A 2.5 mL of blood was drawn and stored at -80°C for subsequent isolation and purification.

Whole blood (IGRA)

The QuantiFERON®-TB Gold Plus (QFT-Plus, 4th Generation) assay utilized specialized blood collection tubes containing Nil control, Mitogen control, TB1 antigens, and TB2 antigens. The blood collection tubes were required to be at room temperature (17-25°C) before and during blood collection. 1 mL of whole blood was then collected into each tube and processed following the standard protocol. Subsequently, 300 µL of plasma was extracted from each tube. To facilitate storage, 100 µL aliquots were taken from each tube and preserved at −80°C.

Whole blood (DNA)

4 mL of whole blood was collected using BD Vacutainer® EDTA tubes from both adults and children. Within 8 hours of collection, the sample was aliquoted, with 1 mL of whole blood allocated into each cryovial, up to four aliquots prepared and stored at -80°C for future analysis (Genotyping and other genetic analysis).

Whole Blood PBMC and Plasma

Whole blood was collected using BD Vacutainer sodium heparin tubes for peripheral blood mononuclear cells (PBMCs) preparation. For adults and children ≥ 5 years old, 8-10 mL of whole blood was collected; while for children < 5 years of age, 4-6 mL of blood was collected. The samples were processed within 6-8 hours of collection, following the RePORT PBMC SOP. The PBMC processing and storage were performed by the standard protocol.

The whole blood separation of PBMCs was conducted using the manual density gradient media overlay method. After separation, 1 to 4 aliquots of 1.0-1.5 mL each were prepared and transferred to 2 mL cryovials. Additionally, the collected plasma was aliquoted in 200 µL aliquots, with up to four aliquots prepared. All aliquots were stored at -80°C for future use.

Saliva

Following proper saliva collection and handling procedures. For Adults and Children ≥ 6 years, a Saliva Collection Aid (SCA) and a 50 mL Conical Tube were used for specimen collection. SalivaBio Children's Swab (SCS) should be used for saliva collection in children under 6 years old, while for infants less than 6 months of age, use the SalivaBio Infant's Swab (SIS). Upon

receipt, 1.5 mL of saliva was aliquoted into each cryovial, with up to four aliquots prepared. Each cap was tightly sealed, and the samples were stored at -80°C.

Urine

A 10 ml clean-catch, mid-stream urine was collected in a sterile, disposable, screw-cap, polypropylene collection cup, and Urine specimen collection bag for pediatric urine samples following the specimen collection and transport guidelines. Upon receipt, the urine was thoroughly mixed before aliquoting. 1.5 mL of urine was aliquoted into each cryovial, with up to four aliquots prepared. Each cap was tightly sealed, and the samples were stored at -80°C.

Mtb isolates

Upon receipt, sputum specimens for culture were promptly processed in a BSL-3 cabinet. Processing included AFB microscopy, LJ solid culture, and MGIT liquid culture. Following the RNTCP guidelines LJ plates were inoculated with decontaminated sputum sediment and positive cultures were confirmed using ZN staining and sub-cultured on blood agar plates (BAP) to confirm purity.

Similarly, MGIT cultures were inoculated and monitored for positivity according to the guidelines of the FIND BD MGIT manual using appropriate algorithms. Susceptibility testing was conducted on positive MTB cultures following the guidelines of RNTCP. Well-grown MTB cultures were sub-cultured on an egg-based medium slant or plate (e.g., LJ, 7H10, or 7H11) and processed. The colonies were homogenized in 5 mL of Middlebrook 7H9 Medium (supplemented with 0.5% glycerol). Finally, 1 mL of aliquots were transferred into 2 mL cryovials, with up to 4 aliquots per specimen stored at -80°C for long-term preservation.

Sputum

Sputum specimens were collected in sterile 50 mL conical tubes, aiming for 3-5 mL for analysis. Single-expectorated specimens were preferred, following standard procedures for collection and handling. For children under 5 or individuals unable to expectorate, alternative methods like sputum induction or aspiration were employed, adhering to established guidelines to ensure high-quality specimen collection. Sputum specimens intended for both testing and storage were pooled and processed immediately upon receipt at the laboratory.

For long-term storage, specimens were treated with Sputasol dithiothreitol (DTT) to liquefy the sample and aid in aliquoting for storage. A low concentration of DTT (final 0.01%) was used in the sample to enable analysis for cell wall components and cytokines. Following the protocol for Sputasol preparation and treatment of sputum specimens, the appropriate volume

of Sputasol was added to the sputum specimen and treated accordingly. Finally, 0.5 mL of aliquots were transferred into 2 mL cryovials, with up to 4 aliquots per specimen. Each cryovial was tightly capped, sealed, and stored at -80°C.

Extracted host RNA

100 – 150µg/vial of extracted RNA from PAXgene Blood RNA tubes was stored at –150°C. This ultra-low temperature ensured the stability of the RNA for long-term storage. Properly labeled and sealed storage tubes were used to prevent contamination or loss during storage. When the RNA was needed, it was thawed carefully to preserve its quality.