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The tuberculosis Molecular Bacterial Load Assay (TB-MBLA)

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UNITE4TB 2ND DEC 2025

RESULT INTERPRETATION GUIDE

True positive for Mtb

1. CT or Cq for Mtb less or equal to 30 (RotorGene PCR machine), 33 (Roche LightCycler 480, AriaDx), 35 (CFX96, Quantistudio & other applied biosystems platforms.
2. Positive control (PCR rexn control) is positive (CT or Cq with the ranges in 1 above).
3. EC is positive (CT or Cq with the ranges in 1 above).
4. NoRT control is negative or has CT/Cq that is more than 5 CTs above the RT+ reaction.
5. Negative control is negative or has CT above the cut offs in 1 above.

True negative for Mtb

1. CT or Cq for Mtb is negative (no amplification) or above 30 (RotorGene PCR machine), 33 (Roche LightCycler 480, AriaDx), 35 (CFX96, Quantistudio & other applied biosystems platforms.
2. Positive control is positive (CT or Cq as in the true positive).
3. EC is positive (CT or Cq with the ranges as in the true positive).
4. NoRT control is negative or has CT/Cq that is more than 5 CTs above the cut offs in one above.
5. Negative control is negative or has CT above the cut offs in 1 above.

Invalid

1. Mtb and EC reactions are all negative.
2. Positive control is negative.
3. NoRT control is positive (equal or less than 5 CTs above the RT+ reaction). DNA removal failed – repeat the DNA removal and run PCR again).
4. Negative control is positive (has CT within the cut offs of a positive reaction. Cross contamination in the water used for RNA extraction or PCR prep. Discard any leftover water aliquots and repeat the process with clean water.

Summary Table of Interpretation

M. tuberculosis	EC	Result
+	+	+
+	_	+*
_	+	_
_	_	Invalid

+ = Positive shown by Ct or Cq from the RT+ reaction.
- = Negative shown by no Ct or CT higher than cut off for a positive reaction.
* = The Mtb presence result is positive, but the result may not be used for quantitative analysis or data normalization.
> Bacterial load from negative or Ct/Cq above the cut off is set at zero.
> Lowest CT/Cq is ideally expected not to be below CT/CQ of 5 equivalent to bacterial load of 10^{10} CFU/ml.

The TB-MBLA summary

- **Detects and quantifies viable *Mycobacterium tuberculosis* bacilli using 16S rRNA as surrogate for colony forming units (bacterial load)**
- **The MBLA consists of three main steps:**
  - Isolation of RNA from *Mycobacterium tuberculosis* cells
  - Quantitative PCR of the extracted RNA
  - Conversion of the PCR output (Ct) into bacterial load (eCFU/ml). eCFU stands for estimated colony forming units per mL.