Enhanced confirmative diagnosis of tuberculous pleurisy through multiplex droplet digital PCR detection of *Mycobacterium tuberculosis* cell-free DNA in pleural effusion

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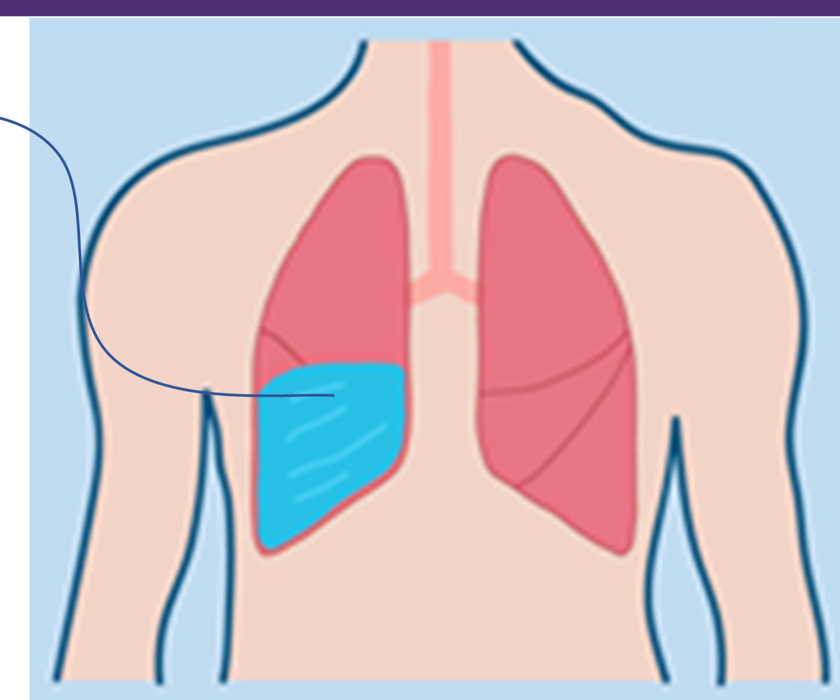
INTRODUCTION

Tuberculous pleurisy (TBP) is a leading etiology of **exudative pleural effusion** in tuberculosis-endemic regions and poses significant diagnostic challenges due to its paucibacillary nature in pleural fluid. This study aims to characterize MTB-derived cell-free DNA (cfDNA) in pleural effusion and to develop an optimized molecular assay to improve TBP diagnostic accuracy.



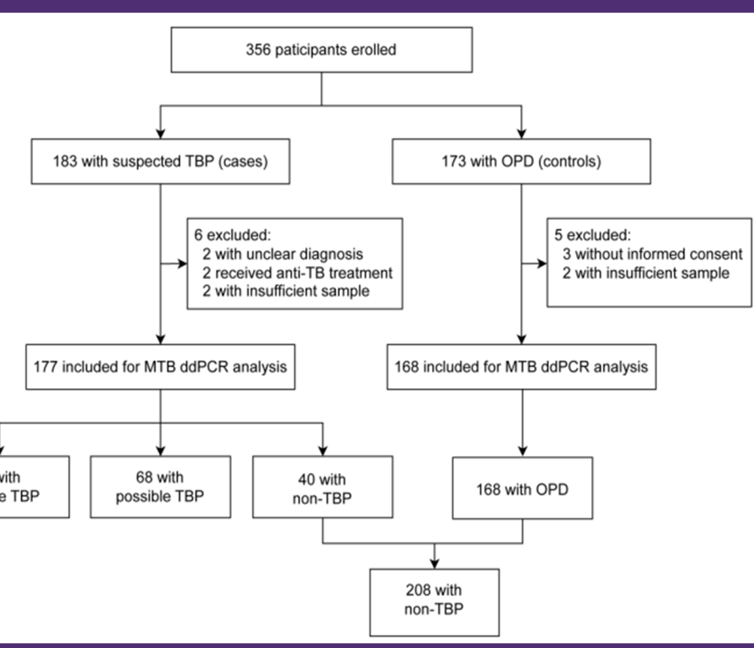
METHODS

Study participants

Flow diagram of study participants.

TBP: tuberculosis pleurisy.

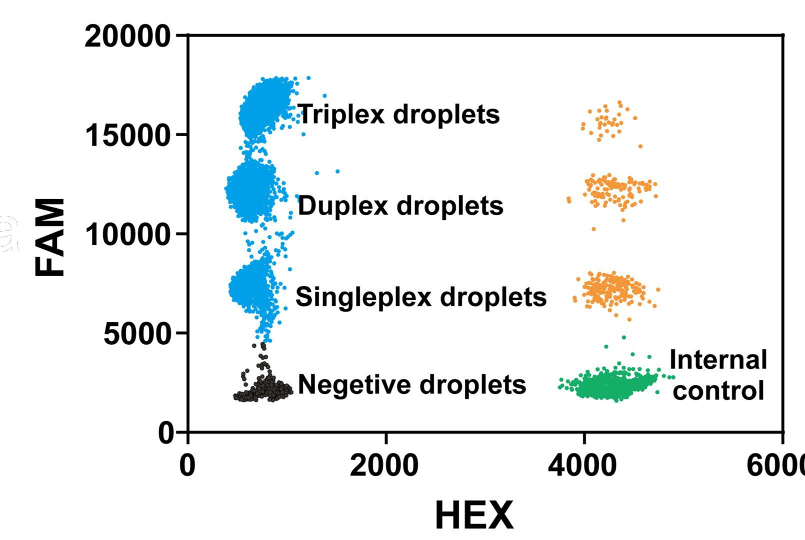
OPD: other pulmonary diseases



Multiplex ddPCR

Multiplex ddPCR assay features:

- Targeting 2 insertion sequences (*IS6110* & *IS1081*)
- Ultra-short amplicons (49-59 bp)



RESULTS & DISCUSSION

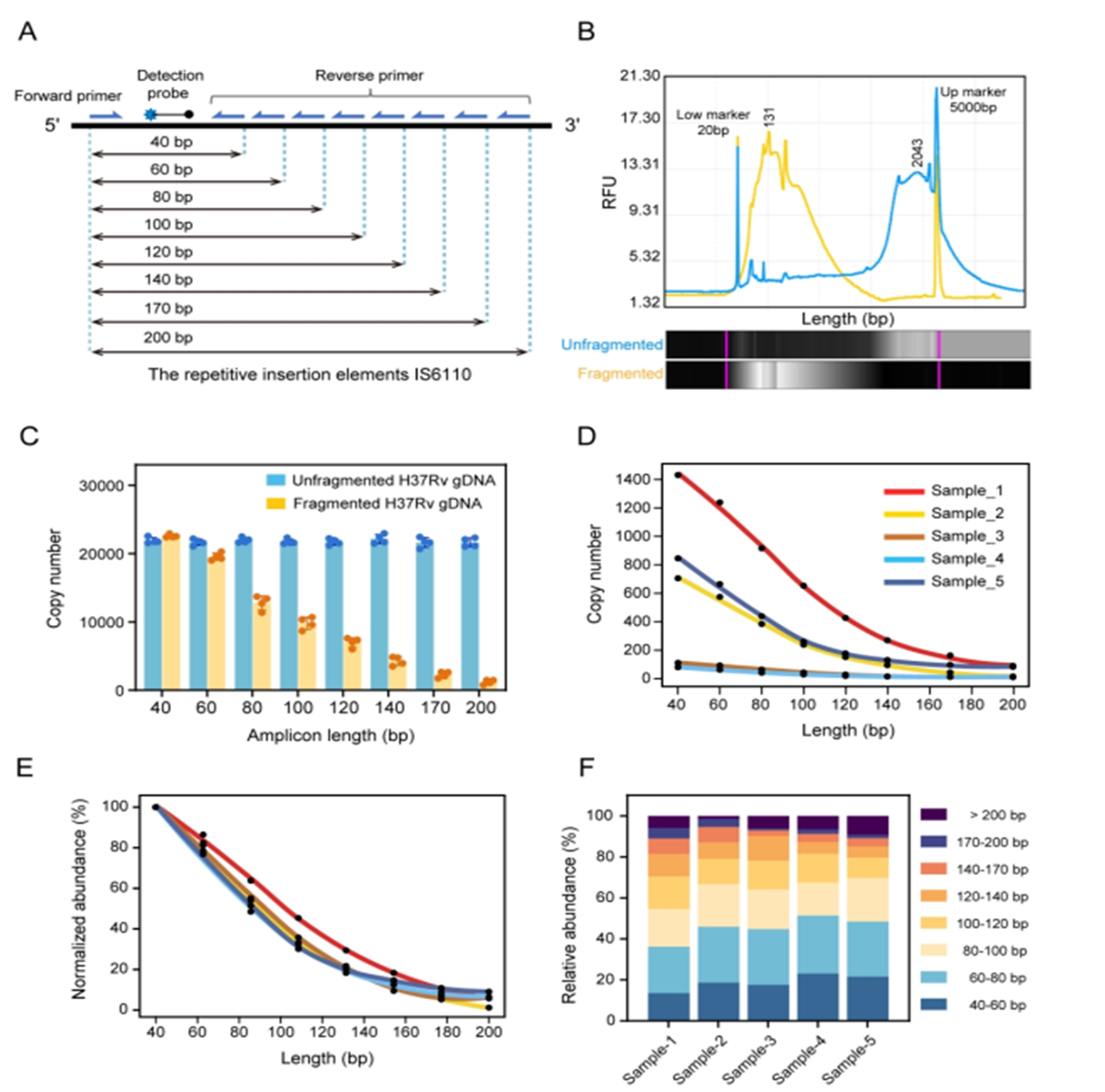


Figure 1. Characteristics of TB cfDNA in Pleural Effusion. (A) Schematic representation of the 40 bp to 200 bp amplicons within the IS6110 region. (B) Template length assessment using capillary electrophoresis: unfragmented H37Rv gDNA (yellow line) and fragmented H37Rv gDNA (blue line). (C) Detection of unfragmented and fragmented H37Rv gDNA by the eight PCR with amplicons size between 40 to 200 bp. (D) The copy number detected using the eight ddPCR for the pleural effusion samples from five definite TBP patients. (E) Normalization of the detected copy number from the samples using the eight PCR. (F) Apparent size distribution of the MTB cfDNA in the patients at different size.

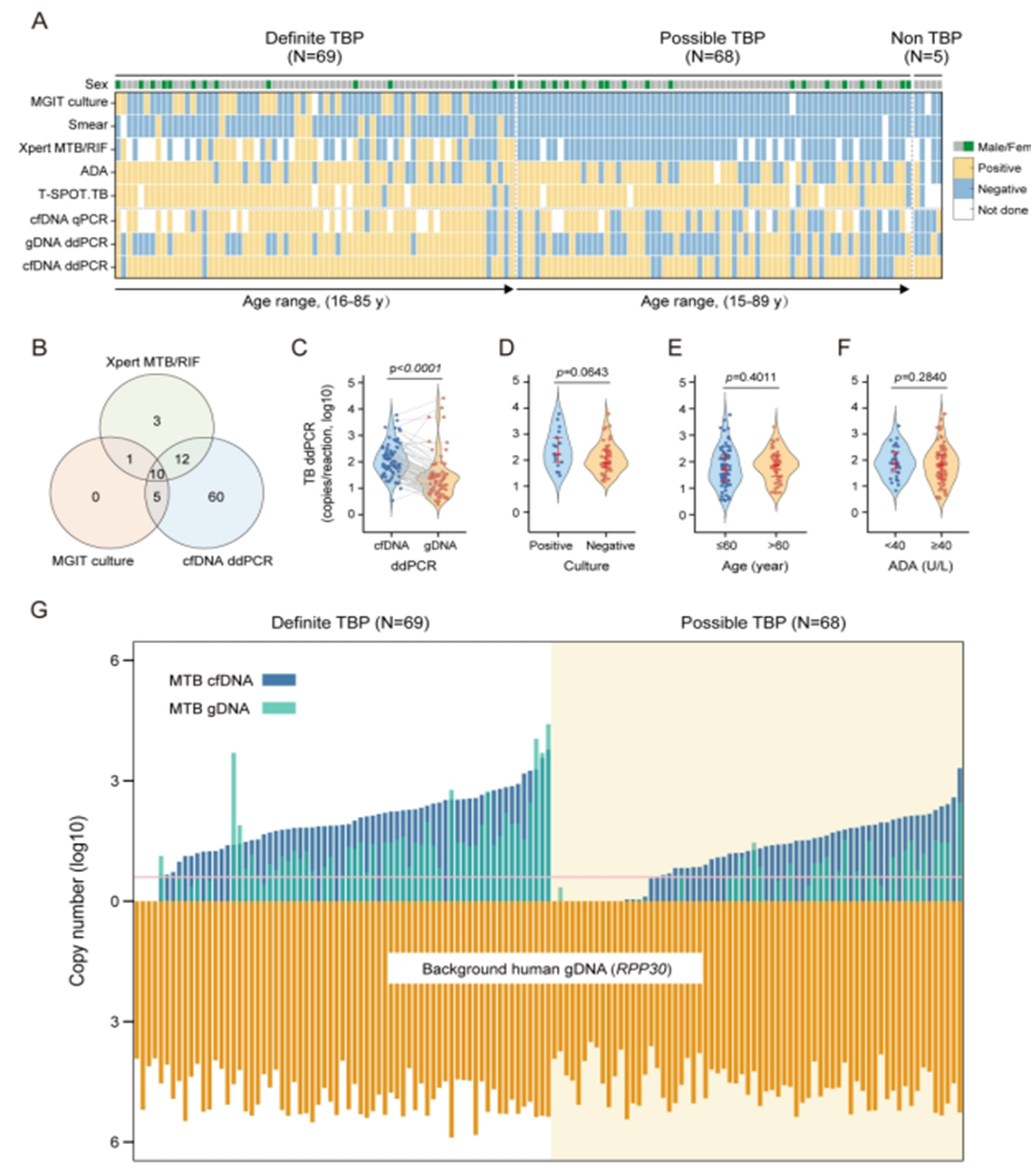


Figure 2. Performance of MTB cfDNA ddPCR assay in patients with tuberculous pleurisy. (A) Detection results of MGIT culture, smear, Xpert MTB/RIF, adenosine deaminase (ADA), T-SPOT.TB assay, cfDNA qPCR, gDNA ddPCR, and cfDNA ddPCR in patients with definite tuberculous pleurisy (TBP), possible TBP, and non-TBP cases. The figure includes all definite and possible TBP cases and five non-TBP patients who had positive cfDNA ddPCR results, (B) and others are omitted.

CONCL.

Our findings establish MTB cfDNA as the predominant nucleic acid form in tuberculous pleural effusion. The optimized multiplex ddPCR platform, leveraging multi-copy targets and fragment-length-adapted amplification, achieves improved diagnostic sensitivity without compromising specificity in a high-prevalence TB setting.

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