Extract from used Xpert MTB/RIF Ultra cartridges is useful for accurate second-line drug-resistant tuberculosis diagnosis with minimal *rpoB*-amplicon cross-contamination risk

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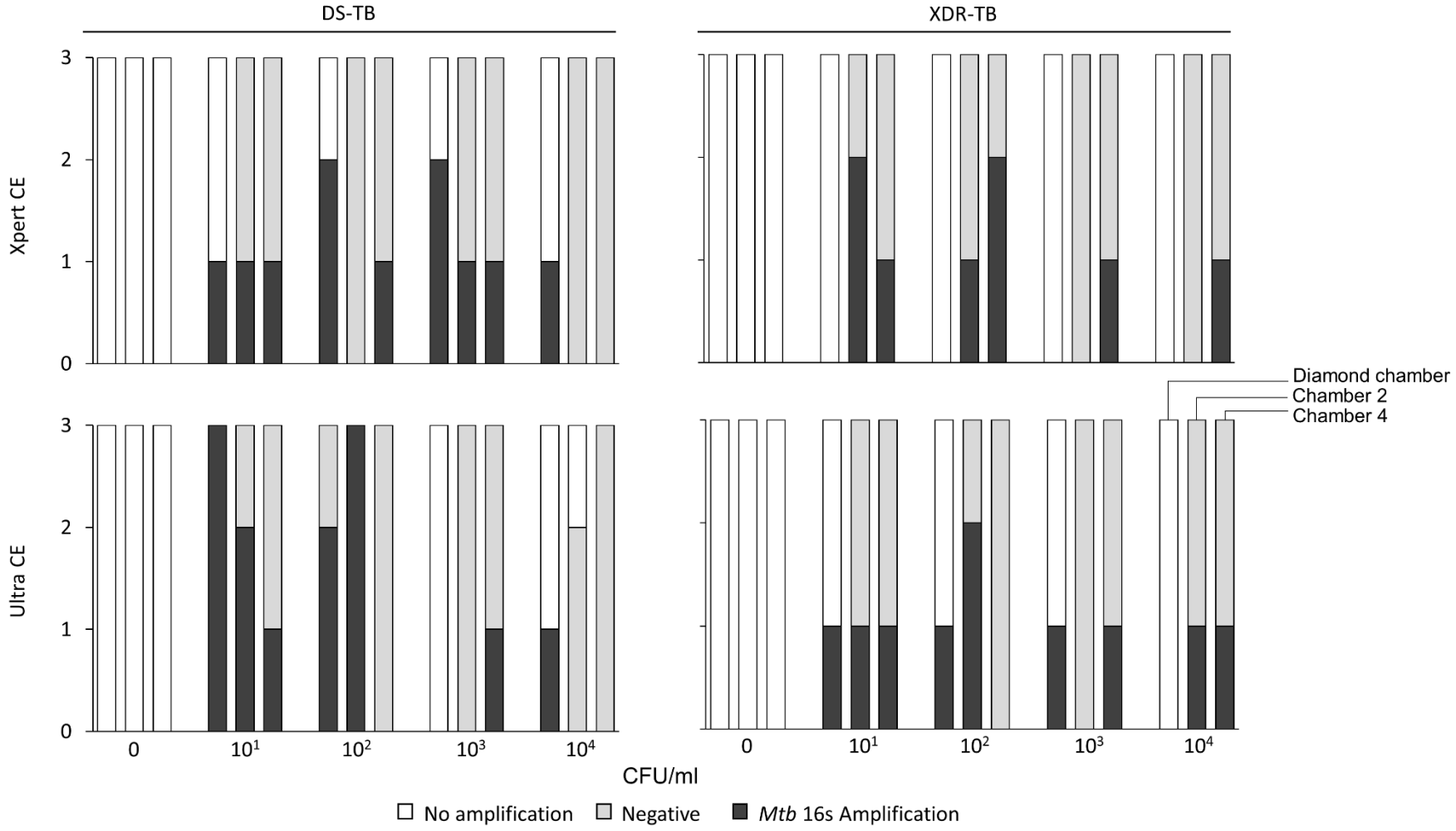
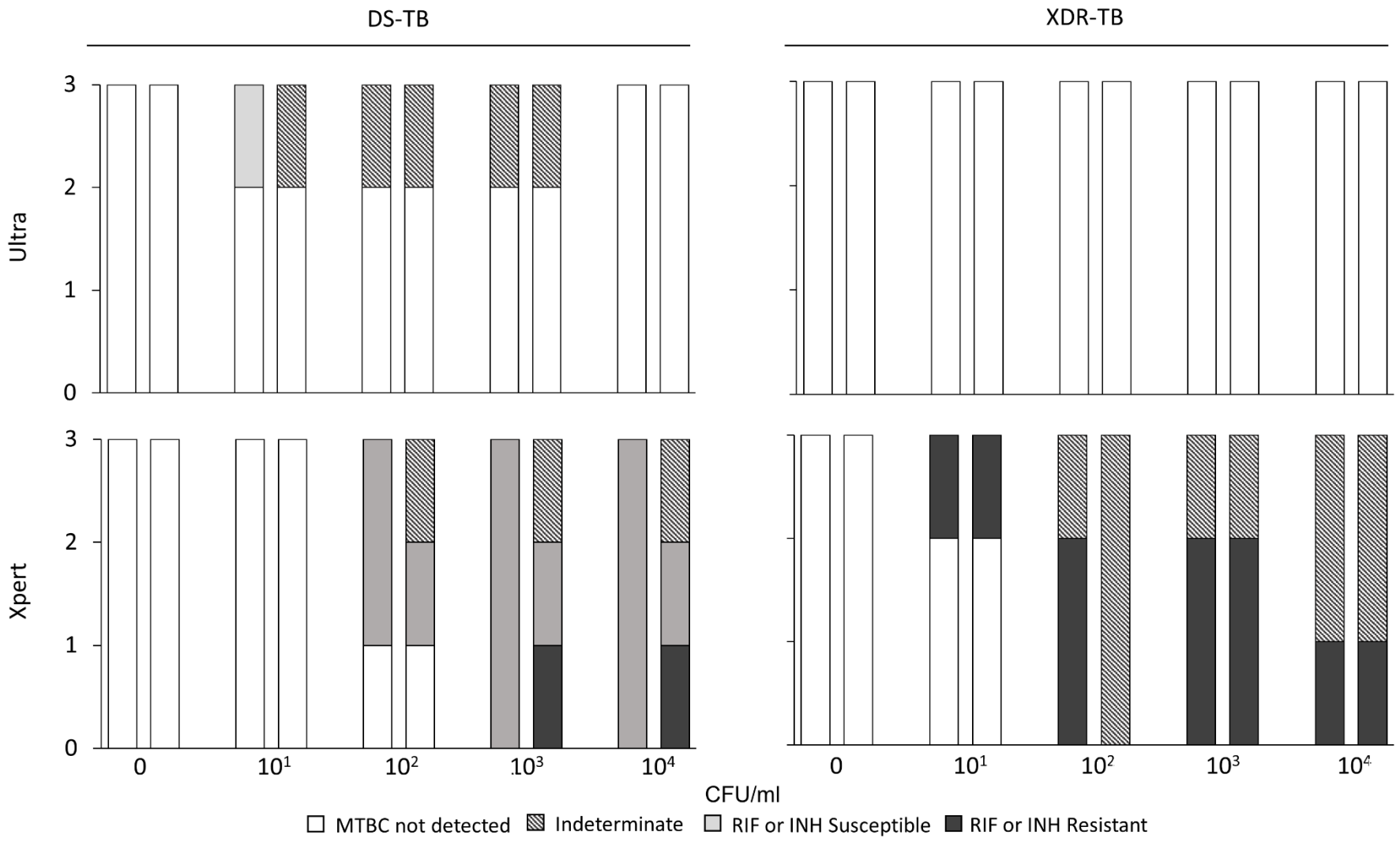
Table S1: V4 region *Mtb* specific 16S rRNA primers and thermocycling conditions

Mtb Forward primer	5'-GTGCCAGCAGCCGCGGTAA-3'
Mtb Reverse primer	5'-GGACTACCAGGGTATCTAAT-3'
Thermocycling conditions (Bio-Rad CFX-96 Real-Time PCR machine)	95°C for 5 minutes 35 cycles of 95°C for 5 seconds 60°C for 30 seconds.

Figure S1


DS-TB
XDR-TB
Xpert CE
Ultra CE
0
1
2
3
Diamond chamber
Chamber 2
Chamber 4
0
10¹
10²
10³
10⁴
CFU/ml
No amplification
Negative
Mtb 16s Amplification

33 **Figure S2:**


DS-TB
XDR-TB
Ultra
Xpert
0
1
2
3
0
10¹
10²
10³
10⁴
CFU/ml
MTBC not detected
Indeterminate
RIF or INH Susceptible
RIF or INH Resistant

Cartridge structure and design

First, sample reagent is mixed with the specimen and 2 ml pipetted into the cartridge [Chamber 1 (C1); ~500 µl typically remaining after test completion]. The cartridge lid is then closed before placement in the GeneXpert machine and the test started. A plunger is automatically inserted into the cartridge centre. This engages a barrel to sequentially draw buffers and reagents into the chambers[1,2]. The plunger first draws the sample mixture into the cartridge base where bacilli (not necessarily alive or intact) are trapped on a filter prior to DNA extraction (Figure 2A)[3]. Wash fluid in Chamber 2 (C2, ~3 ml remaining), is then drawn over the filter and waste is collected in Chamber 3 (C3, ~5 ml remaining). While this wash can mostly (but not completely) remove small DNA fragments, it does not remove large DNA, especially if debris-associated[3-5]. Liquid reagent is then drawn from Chamber 4 (C4, ~500 µl remaining) and a sonic horn applied to lyse bacteria and release DNA[6]. DNA is then drawn into Chamber 5 (C5, no volume remaining) and mixed with reaction beads. This mixture is channelled into the reaction chamber (diamond protrusion at the back of cartridge where dCE is drawn from) where thermocycling and PCR amplification takes place[6].

References:

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5 Blakemore, R. *et al.* Evaluation of the analytical performance of the Xpert MTB/RIF assay. *Journal of clinical microbiology* **48**, 2495-2501 (2010).

6 Cepheid. *Journey Inside the Cepheid GeneXpert® Cartridge - 3D Animation*, 2019).