Lab on a Chip

Electronic Supplementary Information (ESI)

A microfluidic cartridge for fast and accurate diagnosis of *Mycobacterium tuberculosis* infections on standard laboratory equipment

Ana R. Homann,[a] Laura Niebling,[a] Steffen Zehnle,[a] Markus Beutler,[c] Lubov Delamotte,[c] Marie-Christine Rothmund,[d] Daniel Czurratis,[e] Klaus-Dieter Beller,[f] Roland Zengerle,[ab] Harald Hoffmann[g] and Nils Paust[ab]*

S1: Determination of the DNA extraction efficiency in the cartridge.

Decontaminated sputum samples were spiked with two BCG concentrations (50 and 1000 CFU per ml sputum sample). Afterwards DNA extraction was performed with the manual reference protocol and with the cartridge-automated workflow. Each DNA extraction was performed with three replicates. To determine the DNA extraction efficiency in the cartridge, the extracts of the manual reference were compared with the cartridge-generated extracts using the quantitative CE marked diarella MTB/NTM/MAC Kit (Gerbion, Germany). qPCR reactions were run in duplicates on a MIC cycler (Bio Molecular Systems, Australia). To absolutely quantify IS6110 copy numbers, a 10 fold-dilution series of a synthetic IS6110 DNA standard (gerbion, Germany) ranging from 1E6 – 1E0 copies was performed yielding a PCR efficiency and a limit of detection of 99% and 100 copies, respectively. Fluorescence of FAM (IS6110 specific) and HEX (internal control) was measured and recorded after each cycle. Raw qPCR data were analysed with the MIC cycler software (micPCR v2.8.13, Bio Molecular Systems, Australia) while Ct values and IS6110 copy numbers were analysed and plotted using GraphPad Prism 8.4.3. Values are expressed as the mean plus/minus the standard deviation. An unpaired t test was applied to investigate statistical significance in which $p < 0.05$ was regarded as statistically significant.

As shown in Figure S1, the cartridge-automated workflow performed significantly better compared to the manual reference protocol. At low BCG loads of 50 CFU/ml in sputum, the IS6110 qPCR assay results were under or close to the detection limit (DTL) of the assay (100 copies). At a BCG load of 1.000 CFU/ml, the cartridge-automated workflow yielded significantly more BCG target compared to the manual reference protocol, with mean IS6110 copies per 4 µl template of 943.7 (± 385.9) and 225.3 (± 49.12), respectively (n=3, $p<0.05$, unpaired t test). The mean Ct values with DNA extracted in the cartridge and manually extracted DNA were 30.97 (± 0.58) and 33.02 (± 0.20), respectively (n=3, $p<0.01$, unpaired t test).

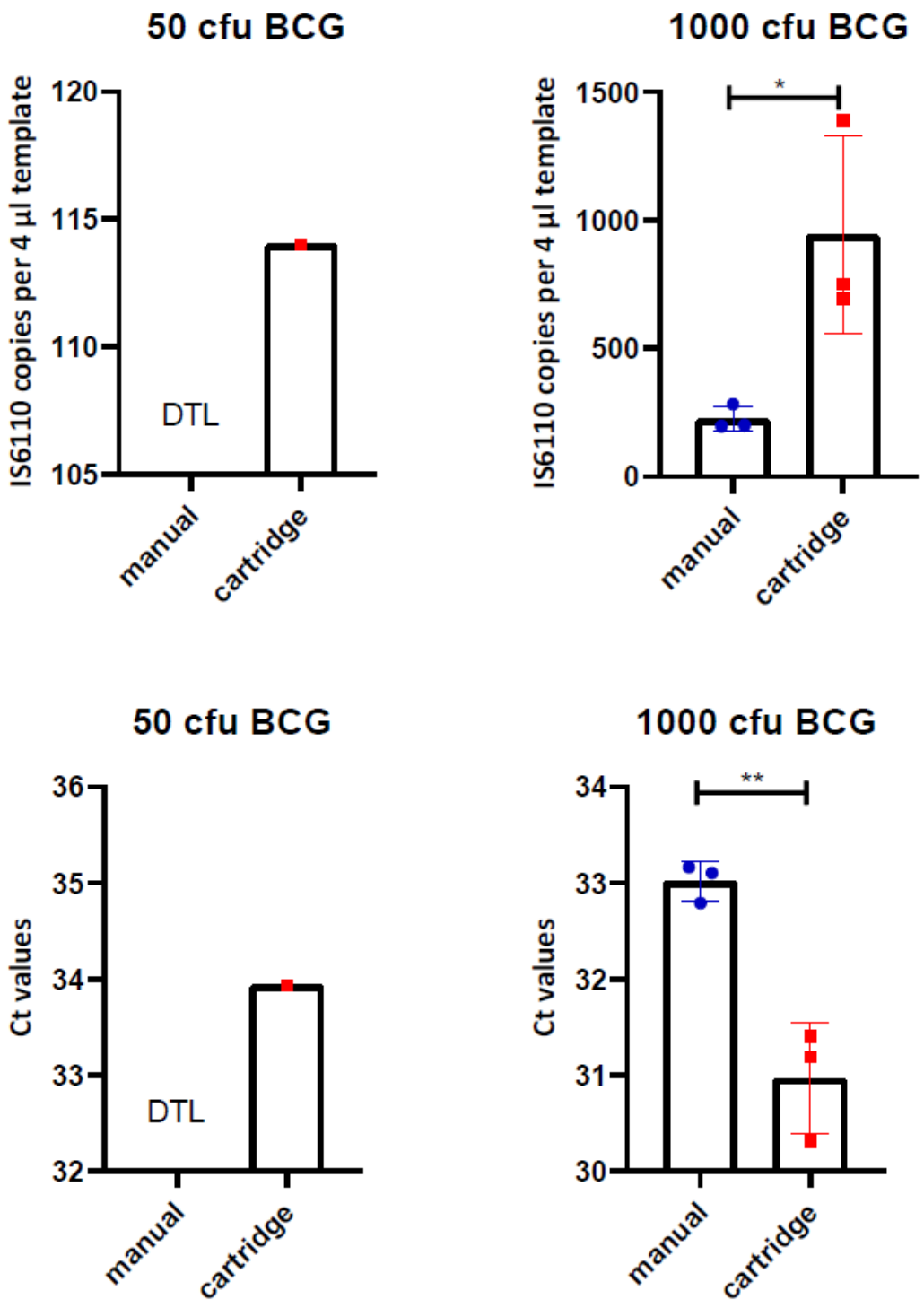

Fig. S1: Results of the IS6110 qPCR running. Extracts of the manual reference were compared to the cartridge-generated extracts regarding the number of detected DNA copies and the associated Ct values for two BCG concentrations (50 and 1000 CFU). Each DNA extraction was performed with three replicates, qPCR reactions were run in duplicates. An unpaired t test was applied (* $p<0.05$; ** $p<0.01$).

S2: DNA gel electrophoresis with PCR-amplified products.

In order to prove that the extracted MTBC-DNA was successfully amplified in the subsequent FluoroType PCR, an agarose (3 % in TAE buffer) DNA gel electrophoresis was performed. Therefore, 4 µl of a DNA extract was mixed with 4 µl 6X DNA Loading Dye (ThermoFisher), with 2 µl ddH2O and with 2 µl GelRed (GeneON) and applied onto the gel. For the DNA ladder 6 µl were used (Ultra low range ladder 10-300 bp, ThermoFisher). The gel was run at 90 Volt for 110 minutes.
Based on the predicted length of the PCR product the MTBC-product was expected at 96 bp whereas the internal control was expected at 70 bp. As shown in Figure S2, specific signals at the expected sizes are visible both for the MTBC-products as well as for the internal control.

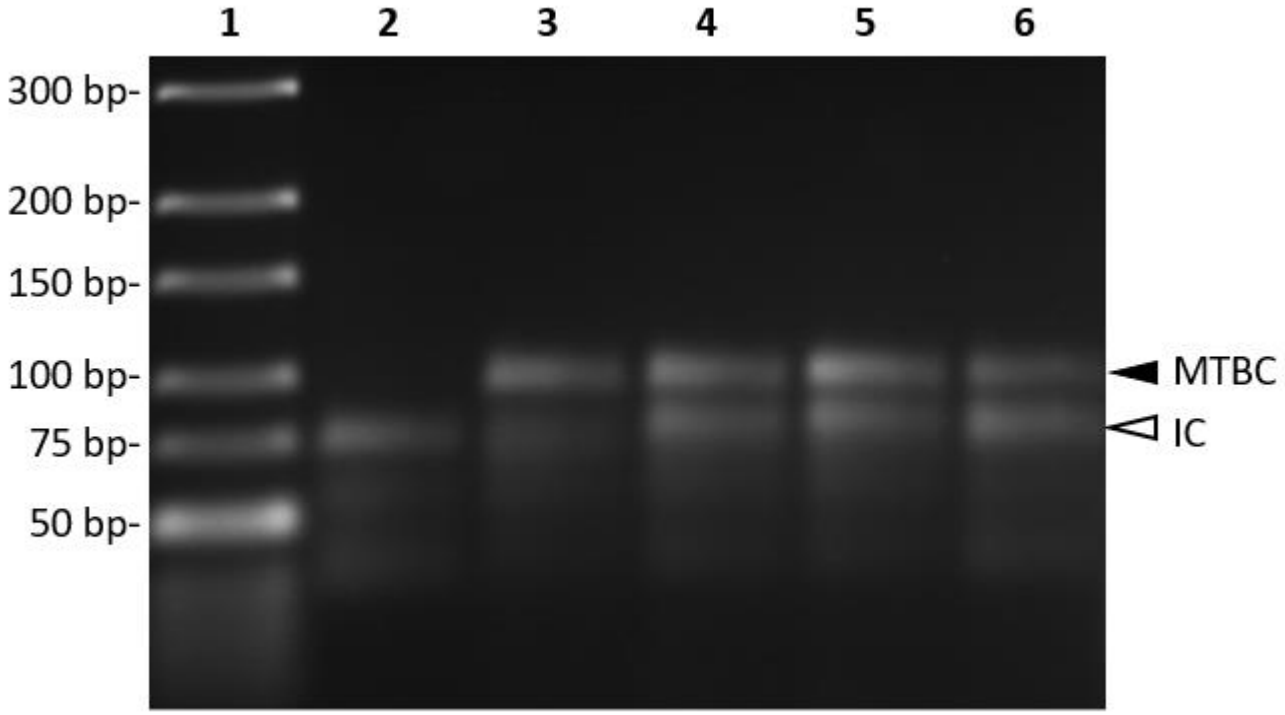

Fig. S2: Gel electrophoresis from PCR-amplified MTBC-DNA. Lane 1: DNA ladder; Lanes 2-6: PCR-products from DNA-extracts generated with the cartridge-automated workflow, where TB-negative sputum was spiked with serial dilutions of BCG prior extraction. Lane 2: 0 CFU of BCG; Lane 3: 10.000 CFU of BCG; Lane 4: 1.000 CFU of BCG; Lane 5: 500 CFU of BCG; Lane 6: 100 CFU of BCG.