Supplementary Material (ESI) for Analytical Methods

Supplementary Information

Mycobacterium tuberculosis Detection via Rolling Circle Amplification

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M. tb. Culturing, genomic DNA extraction, and sample preparation

H37Ra *Mycobacterium tuberculosis* (*M. tb*.) cells were grown in Middlebrook 7H9, supplemented with oleic acid-albumindextrose-catalase (OADC, BD Diagnostics). Middlebrook 7H9 medium (50 ml) was transferred to a sterile, vented flask (75 cm^2). Thawed strain stock (0.2 ml) was added to the bottle with a disposable inoculating loop. The medium bottle cultures were incubated in an incubator at 37 ℃ for 3 weeks to the mid-log phase and harvest by centrifugation. H37Ra cultures were then subjected to 10-fold serial dilutions. These dilutions were plated on Middlebrook 7H11 agar plates and incubation at 37 ℃. Colonies were counted after 2-3 weeks to determine colony forming units per milliliter.

Bacteria were pelleted by centrifugation at 5,000 g for 10 min. The pellet was then suspended in 180 µl buffer ATL. This was then boiled at 95 ℃ for 20 min. Cells were then broken by sonication in a sonic cleaner (Branson 2510) for 15 min. Proteinase K (20 µl) was added, mix by vortexing, and incubate at 56 ℃ for 1 h. DNA extractions were then done with the QIAamp DNA Mini kit (QIAGEN). 3 µg extracted *M. tb.* genomic DNA was incubated at 37 ℃ for 2 h in the presence of 3U of restriction enzyme PvuII and NaeI in a final volume of 30 µl buffer supplied by the manufacturer. Analysis of the genomic

DNA digest was performed by 1% agarose gel electrophoresis and ethidium bromide staining. DNA generated by this method was stored at -20 ℃ and used directly as targets in the detection assay.

M. tb. DNA samples prepared by PCR

To prepare dsDNA targets, Polymerase Chain Reaction (PCR) amplification of *M. tb.* DNA was performed by adding the following components in a sterile micro centrifuge tube: 10 µl 10x pfx amplification buffer, 1 µl deoxynucleotide triphosphates (dNTPs) at 10 mM, 1 µl of 50 mM $MgSO_4$, 1 µl PCR product primer mix at 10 µM, 2 µl genomic template DNA (from the extraction step), 0.5 µl platinum pfx DNA polymerase (Invitrogen), and 34.5 µl autoclaved and distilled water. PCR was carried out in a My Cycler^{TM} thermal cycler(BIO-RAD). PCR was performed by first denaturing samples at 94 ℃ for 4 min, followed by 30 cycles of: 94 ℃ for 15 s, 55 ℃ 30 s, 72 ℃ 60 s. The reaction was maintained at 4 ℃ after cycling, after which samples were stored at -20 ℃. PCR products were then purified with a Purification kit (QIAGEN). Following PCR purification, the DNA concentration was measured by UV spectrometry. This product was serially diluted into many concentrations which were then used to probe the detection sensitivity of the assay. PCR targets were then stored at –20 ℃ until use.

Software for counting target molecules

The developed Matlab program performs three processes. First, amplified fluorescent molecules are identified on a fluorescent image after defining an intensity threshold and nearest-neighbor pixel counting. The intensity threshold was set to be the three standard deviations below the average amplified target molecule intensity. Second, the number of beads and their locations are determined by taking the pixel gradient of the image and applying a Hough transform to brightfield images. Finally, the program determines if a fluorescent DNA molecule resides on the surface of a bead by overlaying two images. If the DNA molecule is on the bead surface, it is counted, and conversely if it is not on the bead surface, it is

discarded. This software allows unbiased, accurate counting of a value for the average number of fluorescent-labeled DNA molecules per bead for a given concentration. All calculations were performed using this software, which is available online at http://chen.seas.ucla.edu/imaging.html.

Specificity for the *M. tb.* assay

Genomic DNA was extracted from *Streptococcus pneumoniae* (ATCC 6303) and *Mycobacterium intercellulare* following the same procedure as *M. tb*. The concentration of genomic DNA from *S. pneumoniae* and *M. intercellulare* after extraction was measured with UV spectrometry to be 190 ng/μl and 8.7 ng/μl respectively. A culture of *M. tb.* bacteria was prepared at a concentration of 2.5×10^6 colony forming units (cfu) per milliliter. From one milliliter of this culture, genomic DNA was extracted and the concentration was measured to be 194 ng/μl. For all three bacteria, genomic DNA was purified and restriction enzyme digested. After digestion, the DNA from these three bacteria was added to the assay to measure the amount of counted molecules per bead for comparison.