Additional File 1

One nanoprobe, two pathogens: gold nanoprobes multiplexing for point-of-care

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Table S1 Multiplex PCR primers and Au-nanoprobes sequences

Name	Sequence
rpoB FW	5’ GAG AAT TCG GTC GGC GAG CTG ATC C 3’
rpoB RV	5’ CGA AGC TTG ACC CGC GCG TAC ACC 3’
Plasm FW	5’ CAGATGTCAGAGGTGAAATTC 3’
Plasm RV	5’ CATGCATCACCATCCAAGAAATCAA 3’
MTBC probe	Thiol - 5’ GAT CGC CTC CAC GTC C 3’
Plasmodium sp. Probe	Thiol - 5’ GGCGAGTATTCGCGCAAGCG 3’
MTBC Target	5’ TGGAACTATGAGTTGGACGTGGAGGCGATC 3’
Plasmodium sp. Target	5’ GTCAATTCTTTTAACTTTCTCGCTTGCGCGAATACTCGCC 3’
Non-related Target	5’ TTG AGT ATC AAG GTG ATC GCC TCC ACG TCC 3’

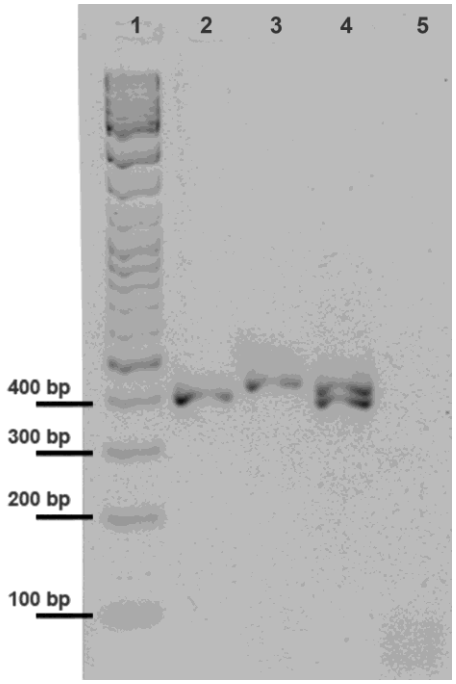

Fig. S1 Electrophoretic analysis on 1% agarose gel: In lane 1 was used Thermo Scientific GeneRuler DNA Ladder Mix; Lane 2 and 3 Multiplex PCR products of *M. tuberculosis* and *P. falciparum*, respectively; Lane 4 Multiplex PCR product of *M. tuberculosis* and *P. falciparum*, Lane 5 negative control of the PCR reaction.

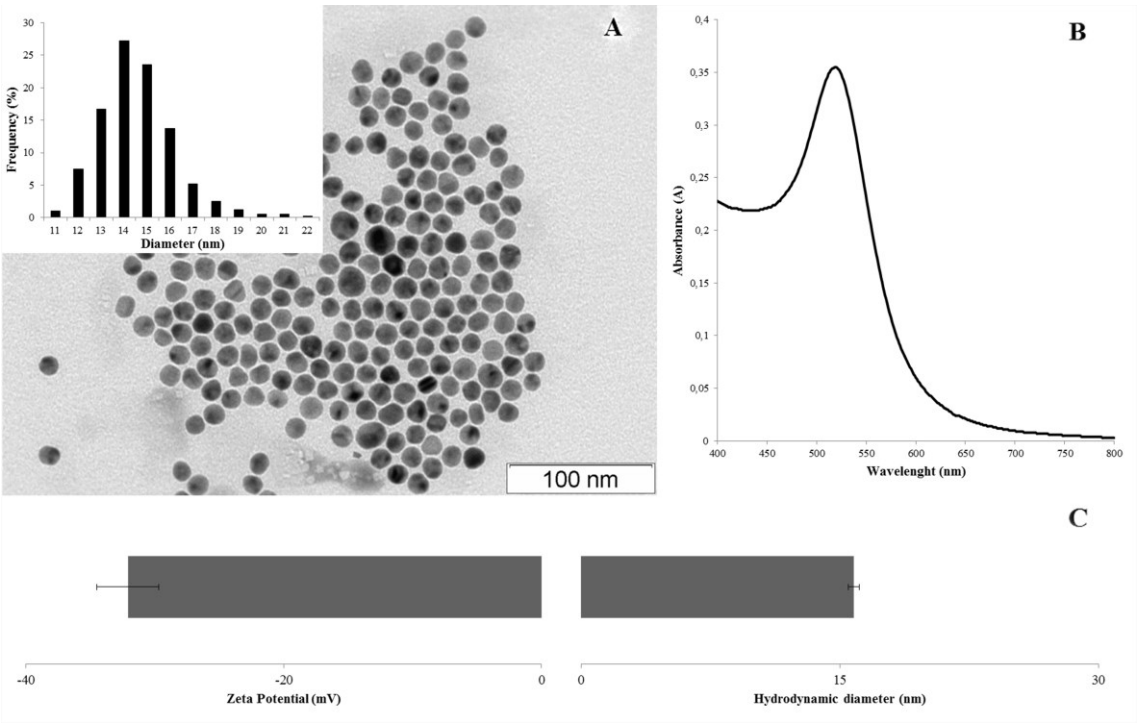

Fig. S2 Physical characterization of the synthesized gold nanoparticles. A) Transmission electron microscopy (TEM) imaging and inset size histogram frequency from ≈ 400 AuNPs counting; B) UV-Vis spectrum of spherical AuNPs, with a characteristic maximum absorption peak, C) Dynamic light scattering (DLS) measurements to define the hydrodynamic diameter of the AuNPs and zeta-potential (ζ-potential) as AuNP surface charge indicator. For DLS it was performed 3 runs per sample with 2000 measurements each and for ζ-potential a total of 5 runs per sample with 250 measurements.

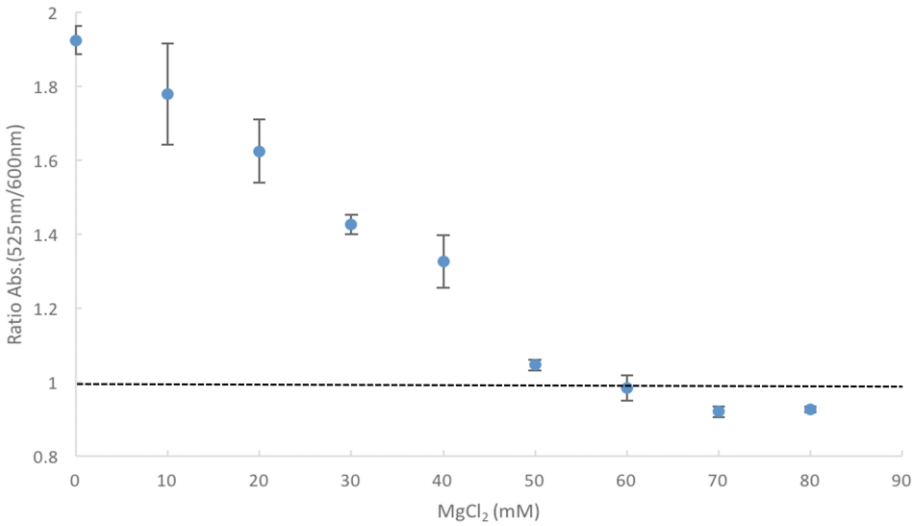

Fig. S3 Aggregation profiles of the synthesized Au-nanoprobes. Aggregation measured as the ratio of localized surface plasmon resonance intensity at 525 nm and 600 nm for increasing salt concentrations ($MgCl_2$). The minimum amount of salt required to cause aggregation was determined based on each Au-nanoprobe aggregation profiles. Ratio <1 was considered for full aggregation, 60mM.

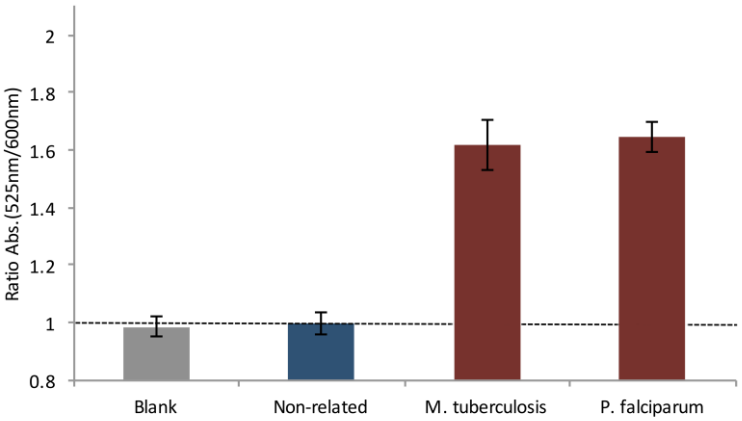

Fig. S4 Single pathogen Au-nanoprobe specificity analysis using synthetic targets. A 1 pmol concentration of synthetic targets (see Table S1) were individually tested with a $MgCl_2$ concentration of 60mM.