Gold-Nanoparticle-Probe–Based Assay for Rapid and Direct Detection of *Mycobacterium tuberculosis* DNA in Clinical Samples

To the Editor:

Many molecular methods have been developed for the direct identification and susceptibility testing of mycobacteria *(1–3)*. Only 2 US Food and Drug Administration–cleared methods are available for direct detection of *Mycobacterium tuberculosis*, the enhanced Direct Test (Gen-Probe) and the Amplicor (Roche). The appearance of multiple-drug–resistant strains and elucidation of the rifampicin resistance mechanism in *M. tuberculosis* have prompted the development of new molecular techniques such as the INNO-LiPA-Rif-TB (Innogenetics), that can simultaneously detect *M. tuberculosis* and sensitivity/resistance to rifampicin *(4)*. However, most molecular tests for *M. tuberculosis* detection are expensive and unsuitable for routine use. Therefore, we sought to develop an inexpensive molecular method for the fast and sensitive detection of *M. tuberculosis*.

Our colorimetric method uses gold nanoparticles for rapid and sensitive direct detection of *M. tuberculosis* in clinical samples with high efficiency after an initial round of PCR. Gold-nanoparticle–based methods have been used extensively for detection of specific DNA and RNA sequences *(5–7)*. We obtained 73 clinical specimens from patients with suspected pulmonary and extrapulmonary tuberculosis with known Ziehl–Neelsen (ZN) acid-fast smear examination results, and we tested these specimens with both the INNO-LiPA-Rif-TB and the functionalized gold-nanoparticle–based methods. All DNA samples were obtained from the Clinical Microbiology Department, Medical University of Lublin, where INNO-LiPA-Rif-TB is used routinely. DNA was isolated from clinical samples by the *N*-acetyl-L-cysteine/NaOH method and purified with a QIAGEN reagent set. For the functionalized gold-nanoparticle *M. tuberculosis* probe, we used a specific oligonucleotide [5′-thiol-GGACGTGGAGGCGATC-3′ (MWG-Biotech)] derived from the *M. tuberculosis* RNA polymerase β-subunit gene sequence (GenBank accession no. BX842574), suitable for mycobacteria identification *(8)*. The

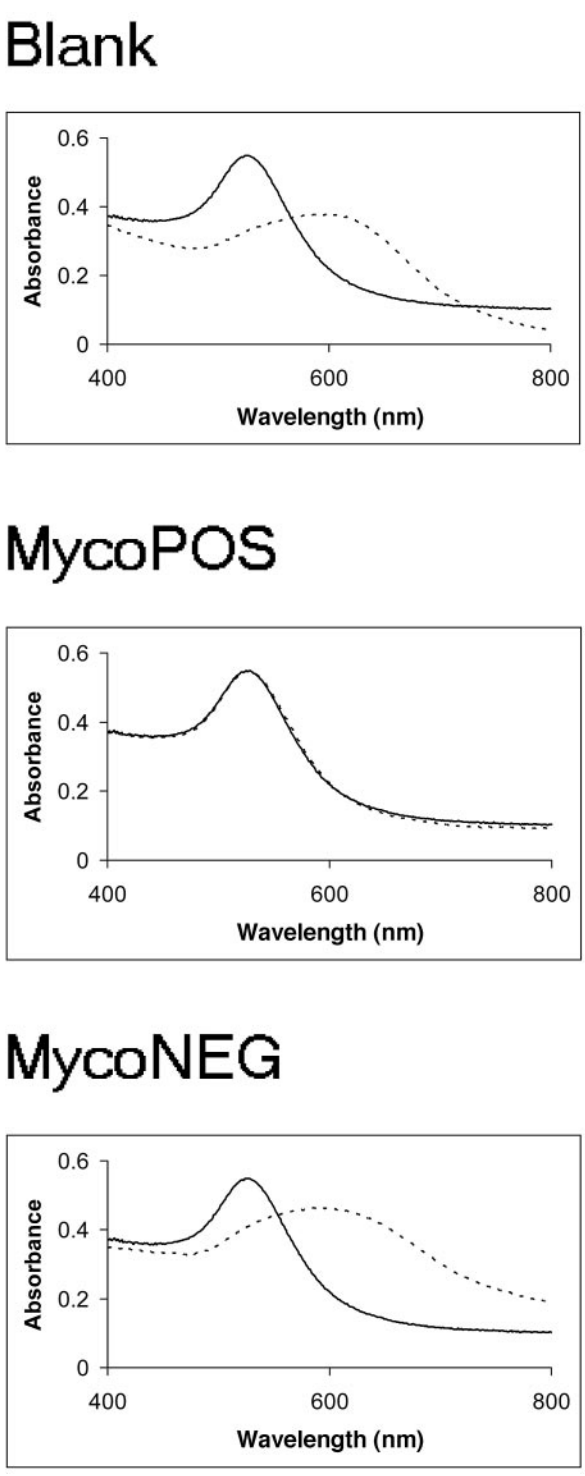

Fig. 1. Gold-nanoparticle-probe–based assay results for *M. tuberculosis* DNA.

Sample color change 15 min after addition of NaCl to a final concentration of 2 mol/L. UV/visible spectra were taken before (*solid line*) and 15 min after (*dashed line*) addition of NaCl. *Blank* refers to nanoprobe and buffer; *MycoPOS* and *MycoNEG* contain DNA from a sample that tested positive or negative, respectively, for *M. tuberculosis* in the INNO-LiPA-Rif-TB assay.

thiolated oligonucleotide was used to produce the nanoprobes as described previously *(6, 7, 9)*.

The nanoprobe solution exhibits a red color because of surface plasmon resonance at an absorbance peak of ~526 nm. At high NaCl concentrations, nanoprobe aggregation in the absence of a complementary DNA sequence turns the solution purple (indicating an absorbance peak shift toward the longer wavelength). In the event of specific probe hybridization to a complementary sequence (i.e., DNA from *M. tuberculosis*), no nanoprobe aggregation occurs, and the solution remains red *(7)*.

We performed the assay by mixing nanoprobe solution (final concentration, 2.5 nmol/L) with test DNA (clinical sample DNA subjected to first-round PCR as described for INNO-LiPA-Rif-TB), for a final DNA concentration of 36 mg/L. After DNA denaturation (5 min at 95 °C), the vial was cooled to room temperature for 30 min to allow hybridization equilibrium. We added NaCl to a final concentration of 2 mol/L and, 15 min later, performed ultraviolet (UV)/visible spectroscopic measurements.

The assay consists of visual/spectrophotometric comparison of the sample with a blank containing an equivalent volume of 10 mmol/L phosphate buffer instead of DNA and with positive (MycoPOS) and negative (MycoNEG) controls containing PCR-amplified DNA that tested positive or negative, respectively, for *M. tuberculosis* with the INNO-LiPA-Rif-TB. The spectrophotometric changes on addition of NaCl for the blank, MycoPOS, and MycoNEG samples for specific *M. tuberculosis* targets are shown in Fig. 1. We observed a purple color change for the blank and MycoNEG samples and no color change for the MycoPOS sample, as corroborated by the UV/visible spectra. (For examples of the color changes, see Fig. 1 in the Data Supplement that accompanies the online version of this letter at http://www.clinchem.org/content/vol52/issue7/).

Previous work indicated that the single strand of the target DNA sequence, and not the complementary DNA strand, preferentially binds to the nanoprobe *(10, 11)*. To determine the method sensitivity, we prepared several MycoPOS samples with total DNA of 0–2.0 μg. At or below 0.75 μg of total DNA, the solution turned purple after addition of NaCl, defining a lower detection limit for the assay. We analyzed 73 clinical specimens: 49 sputum, 6 bronchial washes, 7 pleural effusion, 6 urine, and 5 blood. The acid-fast bacteria in the ZN smear detected 11 positive samples. After a first-round PCR, the nanoprobe assay detected 35 samples positive for *M. tuberculosis*, the same number (100% concordance) obtained with the INNO-LiPA-Rif-TB. Of the 38 samples with negative INNO-LiPA-Rif-TB results, 34 were also negative with the nanoprobe, corresponding to 4 false-negatives in 73 samples. The greater number of positive results obtained with the nanoprobes than with the ZN acid-fast technique suggest that our method may offer more sensitive and reliable *M. tuberculosis* detection.

The nanoprobe assay has a total work-up time of 2 h, and the reaction is performed in a single tube, which reduces carryover contamination. The method is inexpensive and can be performed with simple laboratory equipment, a PCR thermocycler and spectrophotometer, with visual detection made possible by a sharp color change. The overall cost was less than US $0.35 per sample (including first-round PCR), which is considerably less than for other molecular methods.

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Pedro V. Baptista[1*]
Maria Koziol-Montewka[2]
Jolanta Paluch-Oles[2]
Gonçalo Doria[1]
Ricardo Franco[3]

[1] Centro Investigação em Genética Molecular Humana Seção Autonoma de Biotecnologia Faculdade de Ciências e Tecnologia Universidade Nova de Lisboa Caparica, Portugal

[2] Clinical Microbiology Department Medical University of Lublin Lublin, Poland

[3] Rede de Química e Tecnologia Departamento de Química Faculdade de Ciências e Tecnologia Universidade Nova de Lisboa Caparica, Portugal

* Address correspondence to this author at: Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal. Fax 351-212-948-530; e-mail pmvb@fct.unl.pt.

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