

Title	Sensitive and Quick detection of Mycobacterium tuberculosis by isothermal amplification and oxide TFT
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Citation	
Issue Date	2025-09
Type	Thesis or Dissertation
Text version	none
URL	http://hdl.handle.net/10119/20054
Rights	
Description	Supervisor: 高村 禪, 先端科学技術研究科, 修士 (マテリアルサイエンス)

Sensitive and Quick detection of Mycobacterium tuberculosis by isothermal amplification and oxide TFT

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Background and purpose: Nucleic acid detection plays a vital role in diagnosing infectious diseases. Thin Film Transistor (TFT) biosensors provide sensitive and label-free detection for nucleic acid detection. However, in previous TFT measurement setup, ion leakage is observed from single-junction reference electrodes which contaminate and alter the measuring buffer composition and leads to decreased sensitivity. To address this, we introduced a double-junction reference electrode that significantly minimizes ion leakage and maintains stable buffer conditions. Since TFT biosensors operate in low-salt environments to reduce Debye shielding and work with small sample volumes, even minor disturbances can affect measurement accuracy. Additionally, the impact of electrode placement was investigated, as small variations significantly affect ion distribution in low-salt buffers like 0.01X PBS. The optimized system was applied to detect 85B mRNA of Mycobacterium tuberculosis using four different probe types.

Experiment Methodology: The TFT chip was fabricated by optimized conditions of our laboratory. (1) We studied the electrode placement on TFT where we studied placing it on drain electrode and at the center position (Fig.1). (2) Signal stabilization of chips by presoaking them into buffer to reduce signal drift commonly observed in low-ionic-strength environments (Fig.2). (3) The optimized setup was used to study the detection TB using where surface of TFT was modified with probe DNA and signal was compared between the complementary sequence and non-complementary sequence (Fig. 3).

Results and Discussion: By introducing a double-junction electrode and optimizing the electrode position at center and pre-incubation conditions, we significantly improved the stability of current signals. From this we can observe an effective signal in post hybridization (washing in 0.01XPBS) of positive sample for probe 1-4 where DNA probe 3 and probe 4 showed a clear signal difference between complementary (CS) and non-complementary (NC) sequences, likely due to effective detection within the Debye length range.

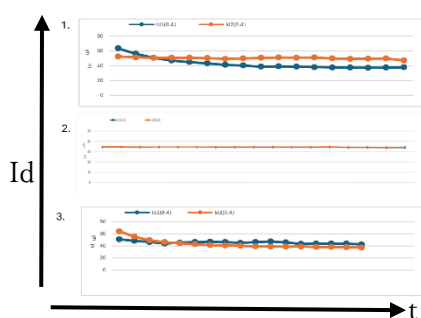


Fig 1. Id-V graph of (1) drain1 (2). center (3). drain

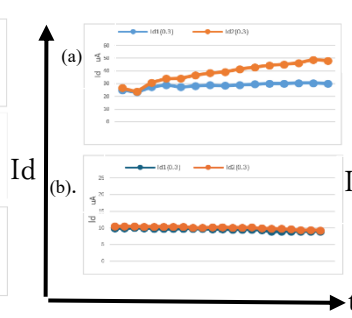


Fig 2. Id-V graph of (a) without soaking of chip. (b) with soaking of chip.

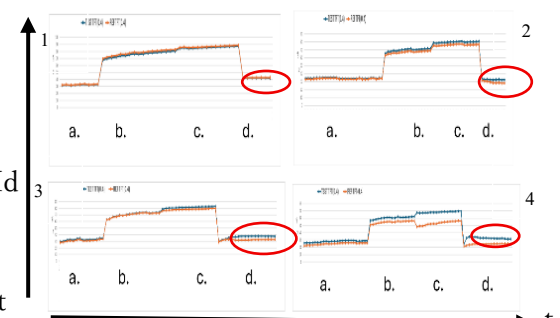


Fig3. Id-V graph of 1.) Probe 1 2.) Probe 2 3.) probe 3 4.) probe 4

Reference

1. Biyani, Radhika, et al. "Development of robust isothermal RNA amplification assay for lab-free testing of RNA viruses." *Scientific Reports* 11.1 (2021): 15997.
2. Wu, Weidong, et al. "Rapid and Highly Sensitive Detection of Leishmania by Combining Recombinase Polymerase Amplification and Solution-Processed Oxide Thin-Film Transistor Technology." *Biosensors* 13.8 (2023): 765.

[Keywords] Oxide Thin Film transistor, RICCA, Tuberculosis