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Simple Processing of Formalin-Fixed Paraffin-Embedded Tissue for Accurate Testing with the Xpert MTB/RIF Assay

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Extrapulmonary tuberculosis (TB) is a common presentation of TB, accounting for 15% of new TB cases in 2018 (1). Under routine practice, tissue biopsy specimens are often sent for histopathology without concurrent mycobacterial culture or nucleic acid amplification testing (NAAT). This leaves formalin-fixed paraffin-embedded tissue (FFPET) as the only viable specimen type for TB testing in these cases. Although TB NAAT can be performed on FFPET, it requires commercial extraction kits with cumbersome deparaffinization and extraction steps (2, 3).

The Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA) is a widely used, automated sample-to-answer, cartridge-based real-time PCR assay approved by the U.S. FDA for accurate detection of *Mycobacterium tuberculosis* in sputum (4). Studies have also shown high sensitivity of the Xpert MTB/RIF assay on fresh tissue (5). While processing of fresh specimens for testing with the Xpert MTB/RIF assay is relatively simple, a simple processing method for FFPET specimens is lacking, and most studies have shown poor performance for the Xpert MTB/RIF assay with FFPET (6–10). The aim of this communication is to describe a simple protocol for FFPET processing and testing with the Xpert MTB/RIF assay and evaluate its performance on FFPET from patients with suspected TB.

Depending on the size of the tissue in the block, three to five 10-μm sections of FFPET were transferred to a clean 2-ml tube. The scrolls were submerged in 400 μl of buffer ATL (Qiagen GmbH, Hilden, Germany) and incubated at 75°C for 5 min to melt the paraffin. The tube was spun at 6,000 $\times$ *g* for 10 sec to sediment the tissue, and 40 μl of proteinase K >600 mAU/ml (Qiagen GmbH) was added. The tube was vortexed for few seconds at medium speed and incubated for 16 h at 56°C. The tube was then spun at 6,000 $\times$ *g* for 10 sec and incubated at room temperature for 5 min to allow paraffin to solidify on the surface. Using a 1-ml pipette tip, a hole was punched through the surface waxy layer, and the entire liquid phase (~440 μl) was transferred to a new tube containing 1 ml of Xpert MTB/RIF sample reagent. After vortexing for 30 sec, the entire volume (~1.440 ml) was transferred to an Xpert MTB/RIF cartridge and tested per the manufacturer's instructions. Sensitivity and specificity of the Xpert assay was determined using a lab-developed *M. tuberculosis* PCR test as the reference method (2).

In total, 61 FFPET samples sent to the Stanford Health Care Clinical Microbiology laboratory for *M. tuberculosis* PCR testing based on presence of granulomatous inflammation on histopathology with or without acid-fast bacilli were included in this study. Tissue sources included lung ($n = 21$), lymph node ($n = 15$), bone and joint ($n = 5$), genitourinary ($n = 5$), intra-abdominal ($n = 5$), gastrointestinal ($n = 4$), bone marrow ($n = 1$), and other ($n = 5$) samples. FFPET samples were tested with a reference *M. tuberculosis* PCR assay and the Xpert MTB/RIF assay, with the former prioritized for clinical testing. Sensitivity and specificity of the Xpert MTB/RIF assay were 85.7% (36/42)

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and 100% (19/19), respectively. All 6 of the FFPET blocks with false-negative results were reextracted and retested, and 3 tested positive. This suggests that sampling error may have contributed to lower sensitivity with the Xpert MTB/RIF assay. Exhaustion of tissue in the block may also be a contributing factor. Another explanation may be the fact that the reference PCR assay targets the multicopy insertion sequence IS*6110*, whereas the Xpert MTB/RIF assay targets the single-copy *rpoB*. Thus, the Xpert MTB/RIF Ultra assay, which targets IS*6110* and another insertion sequence (IS*1081*), may prove more sensitive when used with our processing protocol. In addition, the lower sensitivity observed with the Xpert assay may be inherent to its extraction methodology. The Xpert MTB/RIF cartridge is designed to extract DNA from intact bacteria. Thus, free *M. tuberculosis* DNA resulting from tissue sectioning may be lost during extraction. The extent to which this contributed to lower sensitivity is unknown.

The FFPET protocol described in this study yielded a higher sensitivity than those reported by other studies using the Xpert assay (6–10). The reason for the higher sensitivity with our protocol is unclear, but it is likely independent of patient sample selection and more related to processing steps and the selection of reagents. The Qiagen buffer ATL used in our protocol is a proprietary tissue lysis buffer for use in purification of nucleic acids. In addition to buffer ATL, we evaluated Qiagen buffer G2, which is the recommended buffer for extraction of nucleic acids from FFPET. Interestingly, only buffer ATL proved effective when FFPET was tested with the Xpert assay in our laboratory. However, we do not know exactly how it contributed to improved sensitivity and whether it can be replaced with an alternative lysis buffer.

In summary, we describe a simple processing method for preparation of FFPET for testing with the Xpert MTB/RIF assay. Availability of this protocol will further expand application of the Xpert assay for simple and rapid diagnosis of TB from FFPET.

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