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Paper Poster Session
Tuberculosis - diagnosis and drug resistance

Validation of a real-time PCR protocol for the discrimination between Mycobacterium tuberculosis complex from nontuberculous mycobacteria in clinical specimens

Fanourios Kontos*[1], Kiriaki Paraskevi[2], Vasiliki Mollaki[3], Sophia Vourli[2], Joseph Meletiadis[4], Loukia Zerva[5]

[1]*Laboratory of Clinical Microbiology, "Attikon" Hospital, Medical School, National and Kapodistrian University of Athens, , Athens, Greece*

[2]*Laboratory of Clinical Microbiology, "Attikon" Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece*

[3]*Hellenic National Bioethics Commission, Athens, Greece*

[4]*Erasmus MC, Department of Medical Microbiology and Infectious Diseases, Rotterdam, Netherlands*

[5]*"attikon" Hospital, Medical School, National and Kapodistrian University of Athens, Greece, Laboratory of Clinical Microbiology, Athens, Greece*

Background: Rapid *Mycobacterium tuberculosis complex* (MTBC) identification allows for prompt treatment initiation and patient isolation, acid fast-positive clinical samples, however, may harbour nontuberculous mycobacteria (NTM). This study aimed to validate a Real Time-PCR (RT-PCR) assay (Shrestha et al, 2003, J. Clin. Microbiol. 41: 5121-5126) which differentiates MTBC from NTM. To this end, clinical samples and isolates from patients with mycobacterioses were examined and results were compared with established commercial assays.

Material/methods: We studied a) 62 clinical isolates grown in MGIT960 tubes and including 16 MTBC and 46 NTM strains (19 *M. avium*, 9 *M. lentiflavum*, 8 *M. intracellulare*, 5 *M. kansasii* and 5 *M. gordonae* representing the most common NTM species isolated locally), and b) 87 acid fast-positive clinical specimens, from which 65 MTBC and 22 NTM strains (10 *M. intracellulare*, 9 *M. avium* and 3 *M. kansasii*) were subsequently isolated. The RT-PCR assay (LightCycler 2.0, Roche Diagnostics) amplified 213bp of the mycobacterial 16S *rRNA* gene, including the hypervariable region A to which FRET (fluorescence resonance energy transfer) probes specifically hybridized demonstrating 100% sequence homology with MTBC, but ≥1-bp mismatches with NTM. Subsequently, melting curve analysis and determination of denaturation temperature (Tm) were performed. As comparators for correct MTBC and NTM species identification, the Genotype MTBDRplus and Genotype Mycobacterium CM and AS assays (Hain-Lifescience) were applied. Cost, time to detection and hands-on time of testing by the RT-PCR assay were recorded.

Results: Among clinical isolates, the RT-PCR assay was 100% sensitive for the detection and correct species identification of all MTBC and NTM strains fully differentiating between them. The Tm of MTBC was 64.7±0.89^{o}C (mean±2SDs), which was unequivocally higher ($p < 0.001$) than that of all tested NTM isolates (54.8±5.3^{o}C). Separate and distinct Tm were obtained for the latter: *M. kansasii* 58.65±1.2^{o}C, *M. avium* 56.7±1°C, *M. intracellulare* 54.06±1.42^{o}C, *M. lentiflavum* 52.12±1.1^{o}C (described for the first time) and *M. gordonae* 49.54±0.52^{o}C. Test performance among the 87 acid fast-positive clinical specimens gave results compatible with those of cultures: all 65 MTBC and 22 NTM strains subsequently recovered from clinical specimens were correctly identified. The test was

completed in less than 60 minutes, hands-on time corresponded to 15 min and reagent cost to 5 € per test.

Conclusions: The evaluated RT-PCR method is a rapid, reliable, and low cost assay which can be applied for testing not only mycobacterial isolates, as originally reported, but also acid fast-positive clinical specimens directly after processing. Among the latter, correct MTBC identification and differentiation from NTM is achieved.