

Diagnosis of *Mycobacterium* spp. infection by PCR: usefulness of paraffin-embedded tissue sections

DNA extracts when fresh biopsy specimens are not available



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Introduction

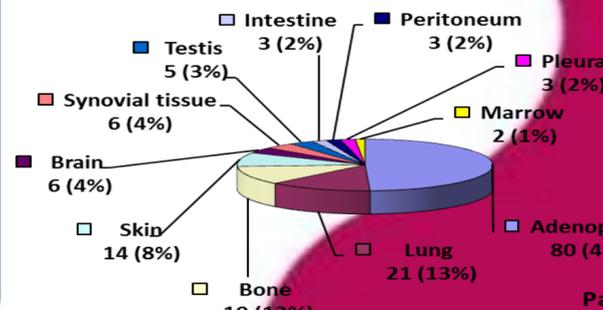
Early diagnosis of micobacteria infection (MI) allows prompt and specific antimycobacterial treatment. Our Microbiology Laboratory often receives paraffin-embedded specimens suspicious of MI from Pathology Department (PD), not submitted for mycobacterial culture. The only option to confirm the diagnosis is to detect mycobacterial DNA by PCR assay. We aimed to analyze the PCR profitability from paraffin-embedded tissue sections DNA extracts *versus* fresh biopsies.

Material/methods

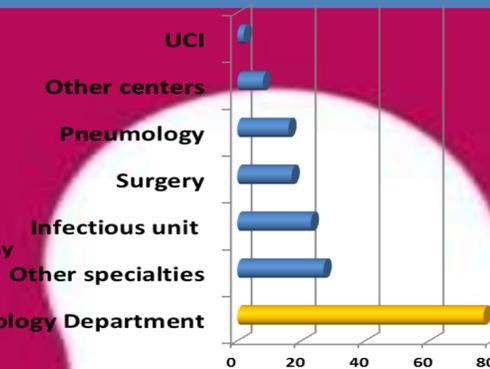
- ✓ From November 2013 to November 2016, 170 biopsy specimens were collected from patients who were clinically suspected to have MI. 108 of which (63.5%) were sent for mycobacterial culture and 62 (36.5%) were submitted from PD, when histopathologic features of MI were present.
- ✓ DNA extraction and PCR assay were performed by FluoroType® MTB v.1.0 (Hain Lifescience) according to manufacturer's standards. In case of paraffin-embedded tissue blocks, DNA extraction was performed by using DNA Sample Preparation Kit (Cobas 4800®, Roche).
- ✓ If high clinical suspicion persisted despite *Mycobacterium tuberculosis complex* (mTB) was not detected or first clinical suspicion was non-tuberculous mycobacteria (NTM) infection, PCR hybridization assay to detect NTM was also performed using GenoType Mycobacterium CM/AS (Hain Lifescience).
- ✓ Clinical-radiological, laboratory, epidemiologic data, suspicious/confirmation diagnosis and previous treatment were reviewed.

Results

- ✓ The study included 170 clinical samples from 106 patients, 60 males (56.6%) and 46 females (43.4%), with the mean age of 44 years. The locations are shown in graphic 1.
- ✓ A granulomatous lesion was observed in 103 biopsies (60.6%) of these, 65 (63%) were necrotizing granulomas.
- ✓ A total of 171 PCR assays were performed (164 mTB-PCR and 7 NTM-PCR).
- ✓ The microbiological and epidemiological results are detailed in graphic 2 and table 1.
- ✓ Of 108 cultured samples, 18 (17%) were mTB-PCR positive (9 positive and 9 negative cultures), and 86 (80%) negative (5 positive and 81 negative cultures). Of 15 positive cultures, there were 3 mTB-PCR false negative (20%) and 3 NTB. The diagnosis in 11 of 21 micobacteria infection, was only made by PCR, with negative culture (Table 1).
- ✓ Of 108 fresh biopsy specimens, 21 (19.4%) obtained a positive result and 87 (80.5%) a negative result by PCR. Of 62 paraffin-embedded tissue blocks, MI diagnosis was confirmed by PCR in 24 (39%) and excluded in 32 (51,6%) according to mTB/NTM PCR negative results, clinical and histopathologic criteria (Table 2).
- ✓ Profitability of PCR for detecting *M. tuberculosis* in paraffin-embedded tissue blocks and fresh specimens were 36.7% and 17.3% respectively.



Graphic 1. Locations.



Graphic 2. Petitioner Service.

Mycobacterial culture	mTB-PCR		NTM-PCR	
	Positive	Negative	Positive	Negative
Positive (N=15)	9	5*	1	0
Negative (N=93)	9	81	2	1
TOTAL 108	18	86	3	1

Table 1. Cultured samples

*5 Positive cultures: 1 *M. lentiflavum*, 1 *M. Intracellulare*, 2 *M. tuberculosis complex* y 1 *M. bovis*

Conclusions

- Performance of mTB/NTM PCR from DNA extracts submitted from PD allows a fast and reliable diagnosis, showing high profitability, even for NTM PCR despite, the technique we used, is not validated for direct sample and reduced number of cases reported.
- Results showed that 53.3% (24/45) of MI diagnosis were confirmed by mTB/NTM PCR from paraffin-embedded tissue blocks, carrying out an early directed treatment.
- We suggest this is a suitable method that should be implemented in laboratories.

Specymen type	mTB-PCR (DNA-extracts N=60; fresh-biopsies N=104)					NTM-PCR (DNA-extracts N=3; fresh-biopsies N=4)	
	Positive	Profitability	Specificity	Sensitivity	Negative	Positive	Negative
DNA-extracts (N=62)	22	36.7%	100%	84.6%*	38	2	1
Fresh-biopsies (N=108)	18	17.3%	100%	75%**	86	3	1
TOTAL (N=170)	40				124	5	2

Table 2. Results.

*2 false negative results (positive *M. tuberculosis* culture), 2 high clinical suspicion cases with TB treatment, 2 no filiated cases.

**3 false negative results (positive *M. tuberculosis* culture), 1 high clinical suspicion case with TB treatment, 5 no filiated cases.