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Title: Do pre-analytical or analytical factors influence the performance of real time PCR tests in the diagnosis of tuberculosis?

Prof. Dr Leila 19895 Antonangelo l.antonangelo@hc.fm.usp.br MD ^{1,2}, Dr. Gabriela 21149 Carnevale ga_bi_gc@yahoo.com.br ², Dr. Lia 21150 Marcal liamarcal@hotmail.com ², Dr. Roberta 21151 Sales roberta.sales@uol.com.br MD ¹, Dr. Milena 21152 Acencio milena.acencio@incor.usp.br ¹ and Prof. Dr Francisco 21153 Vargas pnevargas@incor.usp.br MD ¹. ¹ Pulmonary Division - Laboratory of Pleura, Heart Institute (InCor) - University of Sao Paulo Medical School, Sao Paulo, Brazil and ² LIM 03 - Clinical Laboratory, Pathology Department - University of Sao Paulo Medical School, Sao Paulo, Brazil .

Body: Purpose: The tests used for nucleic acid amplification of Mycobacterium tuberculosis have sensitivity ranging from 11 to 81% in samples of pleural fluid (PF), sputum or bronchoalveolar lavage (BAL). The lowest sensitivities are observed in paucibacillary samples and in samples with pre analytical interferents. The objective of this study is to evaluate whether pre analytical variables influence the diagnostic of tuberculosis by real-time PCR in samples of PF and BAL. Methods: From a pleural transudate sample we simulated a PF sample rich in proteins (>4,5 g/dL), cells (>10.000/mm³) and eritrocytes (>50.000/mm³) and inoculated M. tuberculosis in concentration ranging from 1,5 x 10⁶ to 1,5 x 10¹ CFU/mL. From a non-inflammatory BAL sample we simulated BAL samples with increasing grades of hemorrhage. All the samples were prepared in triplicate. Two extraction (Qiagen and Roche) and detection (Roche and Nanogen) techniques were used for the Real time PCR.

Conclusions: The choice of the extraction/detection methods seems to be more important than samples interferents in the detection of M. tuberculosis in paucibacillary PF and BAL samples.