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Research of *Mycobacterium tuberculosis* complex DNA in paraffin-embedded histological samples by GenoTypeMTDRplus

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Background: The possibility to detect *Mycobacterium tuberculosis* complex DNA in formalin-fixed and paraffin-embedded bioptic samples, by GenoType[®]MTBDRplus (HAIN Lifescience, Germany), is useful to confirm or exclude tuberculosis diagnosis, in absence of microbiological culture. DNA must be researched in samples with a morphological pattern suggestive for tuberculosis disease.

The aim of our study was the evaluation of this test for tuberculosis diagnosis, and the comparison our molecular data with clinical data.

Material/methods: From January 2013 to November 2016, in ASST Niguarda Hospital in Milan, we collected and examined 116 paraffin-embedded histological samples with a morphological pattern suggestive for tuberculosis, owned by 116 different patients in different body sites: 45 bioptic samples from lymph node, 32 from respiratory tract and 39 from other sites.

Each histological sample, after paraffin removal, was exposed to Genomic DNA with iPrep™ Purification Instrument, using a specific kit (iPrep™ChargeSwitch Forensic Kit – Invitrogen) Extracted DNA were after quantified by Spectrophotometric Analysis. They were then analyzed using GenoType®MTBDR*plus* test, following producer's instruction, specially for parameters used for research by direct sample.

Important for the an appropriate execution of test is purity degree of DNA extract, represented by 260/280 Ratio.

For each sample, we collected clinical data significant for tuberculosis disease.

Results: For 18 of 115 samples, it was impossible finding clinical data, so we considered 97 samples. Results of Molecular evaluation and clinical diagnosis, on the basis of available clinical data, are shown in Table 1.

| | | Tuberculosis | NO Tuberculosis |
|--------------------------------|----------|--------------|-----------------|
| GenoType®M TBDR <i>plus</i> | Positive | 71 | 1 |
| | Negative | 4 | 21 |

Table 1: Molecular results and Clinical diagnosis

GenoType®MTBDR*plus* Test showed 94,67% of Sensibility and 95,45% of Specificity.

For 5 samples with discordance between Test results and clinical diagnosis, 260/280 Ratio was less or equal to 1,4.

Conclusions: Results suggest that the amplification method GenoType®MTBDR*plus* applied on formalin-fixed and paraffin-embedded bioptic samples, with a morphological pattern of tuberculosis, could be an efficient contribution at diagnosis, allowing a further association between molecular and morphological data. Obviously, these results must be correlated to clinical signs to formulate a correct diagnosis.

Data of purity degree of DNA extract, represented by 260/280 Ratio, is proved fundamental on test reliability, because values less or equal to 1.4 seem make unreliable molecular results.