MAS-PCR: A Quick Cheap Detection Test for Isoniazid Resistance in *Mycobacterium tuberculosis*

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Background: Every year, approximately 2—3 million people die of tuberculosis. TB control programmes are threatened by the HIV epidemic and increasing rates of drug resistance in many of the most severely affected countries. Although Vietnam has reached WHO targets for TB detection and cure since 2000, the prevalence of TB has not declined. Vietnam has high rates of primary drug resistance, with 25% of new patients in HCMC infected with INH-resistant organisms, which is likely to be a contributing factor to the continued high prevalence of TB. To improve the efficacy of TB control programs in targeting drug-resistant isolates, rapid tests are urgently required. Commercial tests are available but the price is prohibitive in low-income settings. The technique chosen should be simple, inexpensive and accurate.

Methods: Multiplex Allele Specific PCR (MAS-PCR) was developed to detect the two most dominant INH-resistance mutations in *M. tuberculosis*, the first at katG315, and the other at −15 upstream of inhA promoter. Conventional 1% proportion drug susceptibility testing at 0.2 μg/ml was used as gold-standard to compare the sensitivity and specificity of the commercial MTBDR (Hain, Lifescience) and the novel MAS-PCR in 100 consecutive INH-resistant and 50 INH-sensitive isolates from Pham Ngoc Thach Hospital, HCMC.

Results: The detection rate of the MAS-PCR was 90% of phenotypically INH-resistant isolates. The specificity and the sensitivity of the two tests on culture isolates are 100% and 90% with MAS-PCR, 100% and 78% with MTBDR, respectively.

Conclusion: The MAS-PCR developed here is economical, simple and accurate for identification of INH-resistant *M. tuberculosis* from culture. With 100% specificity, it has the potential to be widely used as rapid screening test for INH-resistant TB in areas where the use of commercially available kits is not feasible.

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48.009

Usefulness of Gamma-Interferon Determination in Blood and Cerebrospinal Fluid for the Diagnosis of Tuberculous Meningitis

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Objectives: The study prospectively analyses the different diagnosis methods for tuberculous meningitis available in Infectious Diseases Hospital Iasi, Romania.

October 2007 in our hospital, regarding epidemiological, clinical data and laboratory methods of diagnosis: cerebrospinal fluid examination and culture, tuberculin skin test, radiological assays and quantification of gamma-interferon in blood and cerebrospinal fluid using Quantiferon TBGold assay (QTF).

Results: The patients were divided in 2 groups: one of 17 patients with TB meningitis and one control group of 28 patients with either bacterial (3c), lymphocytic meningitis (17c) or without central nervous system infection (7c). TB meningitis was more frequent in males, with a mean ratio of 2.5. The age of patients varied between 2 and 78 years, with a mean of 30.5 years. Many of the patients were children (35.6%) and 40% were older than 36 years. In 71.1% (32c) of the cases the meningean infection was secondary, disseminated from a primary site, most frequent a pulmonary one (68.7%). We had bacteriological confirmation by culture in 6 cases (13.3%), with a medium of 23.4 days of cultivation time. For the other cases the diagnosis was established by clinical data, cell count and biochemical analysis of CSF, the presence of extrameningean tuberculous infection and positive evolution under antituberculous therapy. TST was performed to all the patients except 3 AIDS patients; it had a sensitivity of 71.4% and a specificity of 62.5%. In whole blood the sensitivity of QTF was 78.57. In CSF the specificity was higher than in blood (96.1 vs 88.4%), having a positive prediction value over 90%. Antituberculous therapy prior to QTF testing had a negative impact, 5 of 6 cases having negative QTF results.

Conclusion: QTF (serum or CSF) had a better sensibility and specificity than TST, CSF direct exam or culture and could be used to establish an early diagnosis of tuberculous meningitis.

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Routine Diagnosis of Tuberculous Meningitis with MODS Assay

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Background: Tuberculous meningitis (TBM) is a devastating condition. The rapid instigation of appropriate chemotherapy is vital to reduce morbidity and mortality. However rapid diagnosis remains elusive; smear microscopy has extremely low sensitivity on cerebrospinal fluid (CSF) in most laboratories and PCR requires expertise with advanced infrastructure and has sensitivity of only around 60% under optimal conditions. Neither technique allows for the microbiological isolation of M. tuberculosis and subsequent drug susceptibility testing. We report here on the routine use of the microscopic observation drug suscep-

tibility assay (MODS) format for diagnosis of TBM in our hospital.

Methods: Four hundred and ten consecutive CSF samples collected from 277 patients clinically suspected of TBM presented at the Hospital for Tropical Diseases, HCMC, between December 2006 and October 2007 were tested by Ziehl-Neelsen (ZN) smear, MODS, Mycobacterium growth indicator tube (MGIT) and Lowenstein-Jensen (LJ) culture. One hundred and sixty-eight samples were from patients already on TB therapy for >1day and 32 samples were excluded due to untraceable patient records. Two hundred and forty-two samples from 226 newly presenting patients included in. 49.6% (n = 112/226) of patients were deemed to have TBM by clinical diagnostic and microbiological criteria (excluding MODS).

Results: Sensitivity by patient against clinical gold standard for ZN smear, MODS, MGIT and LJ were 26.3%, 55.9%, 66.9% and 58.5%, respectively. Specificity of all microbiological techniques was 100%. Positive and negative predictive values for MODS were 100% and 71.3%, respectively for HIV infected patients and 100% and 69.8% for HIV negative patients. The median time to positive was 7 days, significantly faster than MGIT at 16 days and LJ at 31 days.

Conclusions: We have shown MODS to be a sensitive, rapid technique for the diagnosis of TBM in routine use.

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Role of Excretory Urography in Urinary Tuberculosis

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Background: One of the commonest extrapulmonary site for tuberculosis is genitourinary system. Definite diagnosis of tuberculosis is by urine acid fast staining or biopsy specimen, which is time taking and thus the final diagnosis may be delayed. Though the IVU features are nonspecific, presences of multiple patterns suggest tubercular culture or biopsies for confirmation there by possible early initiation of treatment. Here is a retrospective study to analyze the spectrum of imaging findings of tuberculosis affecting the kidney, ureter, bladder on excretory urography.

Methods: The study was conducted retrospectively in a teaching hospital between 2002 and 2006. Proven cases of urinary tuberculosis with excretory urography were collected. Total 50 IVU examinations were reviewed for various imaging findings and three specific imaging patterns. Multiple findings were considered to be present when single finding seen involving multiple sites or co-existing multiple findings.

Results: Most common finding was hydrocalycosis, hydronephrosis or hydroureter secondary to strictureseen in 36 cases out of 50, followed by parenchymal cavities and irregular or moth eaten calyces 6 each, calcifications and scarring in 5 cases each, autonephrectomy in 4 cases and thimble bladder in 12 IVU imaging. Multiple findings with three imaging patterns are noted in 36 (72%) cases. Various imaging pattern includes multiple stricture sites seen in 25 out of 50, a single stricture with one other imaging finding in 7 cases or autonephrectomy with one another findings other than stricture seen in 4 cases.

Conclusion: Spectrum of imaging findings of urinary tuberculosis was seen on IVU in our study. Although each finding is nonspecific, suspicion of tubercular etiology should be made when there are multiple findings with three imaging pattern on excretory urography and tubercular culture or biopsies should be suggested.

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48.012

Direct Detection of *Mycobacterium tuberculosis* Complex and Four Clinically Relevant Mycobacterial Species with Geno Type Mycobacteria Direct Test

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Introduction: The objective of this study was to detect *Mycobacterium tuberculosis* complex and clinically relevant four nontuberculous mycobacteria (*M. avium, M. intracellulare, M. malmoense and M. kansasii*) directly with 23S rRNA-amplification based assay from patient specimens.

Materials and Method: Sputum and other extrapulmonary cilinical specimens obtained from the selected patients who admitted to our center with tuberculous symptoms as a fatigue, slight fever, loss of appetite, unintended weight loss, night sweating, coughing three or more weeks and hemoptysis during January 2007- January 2008. Cilinical specimens accepted from the only patients who were not treated or treated less than 7 days with anti-tuberculous drugs and who did not receive such a treatment within the last 12 months. Existence of the RNA belongs to the five mycobacteria species (*Mycobacterium tuberculosis* complex, *M. avium*, *M. intracellulare*, *M. malmoense* and *M. kansasii*) investigeted with reverse-hybridisation method with three steps. Mycobacterial RNA isolation from decontaminated sputum and other clinical specimens using Magnetic Beads Capture technique(Magnet Separator version 3 Hain Lifescience GmbH, Germany), amplification based on the Nucleic Acid Sequence Based Amplification technique (NASBA reaction) with thermal cycler (Bioer XP Cycler, Japan) and finally the amplification products are selectively hybridized to the Geno Type Mycobacteria Direct test stribs Ver 4.0 (Hain Lifescience GmbH, Germany) with Auto-LiPA enstrument (Tecan ProfiBlot T48, Austria). Five different mycobacteria strains identified with only one detection strib. All of the investigated samples with Geno Type Mycobacteria Direct Test method were also stained with EZN and examined with microscobically. All the patients evaluated with symptoms, physical examination, osculta-tion of the chest with stetescope, chest X-ray screening and other laboratory findings(CBC, ESR, CRP).

Results: Of 144 patient specimens 58 were sputum (81%), 26 were pleural liquid (18%), 2 were cerebro spinal fluid (CSF) (1%) and mycobacteria strains were detected in 30 ones (21%).Of 30 detected mycobacteria strains 28 were identified as *Mycobacterium tuberculosis* complex (93%) and