**Evaluation of Commercial Real Time PCR and Immunochromatography Techniques in Laboratory Diagnosis of Tuberculosis**

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**Abstract**

This study is a diagnostic evaluation study to real time PCR and Immunochromatography compared to conventional microbiological techniques (represented by Acid fast smear and culture) in laboratory diagnosis of tuberculosis. Whole blood and sputum samples were collected from 136 tuberculosis patients, 124 of them were pulmonary tuberculosis and 12 of them were extrapolmonary tuberculosis in addition to 102 healthy control subjects at consultant clinic for respiratory diseases in Hilla – Babil province-Iraq, during the period from May 2010 to June 2011. Real-time PCR kits, Immunochromatography rapid test in addition to acid fast smear and Lowenstein-Jensen culture were used to diagnose the tuberculosis cases. The results show that the sensitivities of the AFB smear, culture, RT-PCR and ICG were 74%, 43% 88% and 24%, respectively. The sensitivity of 99% was obtained by using RT-PCR and/or AFB smear as a diagnostic criteria. The specificity of AFB smear, culture, RT-PCR was 100%, while the specificity of ICG was 98%. The using of both AFB smear and RT-PCR as diagnostic tests raised the sensitivity to 99% compared with the using of the AFB smear, culture and ICG as a single test.

**Keywords:** Immunochromoatography**,** laboratory diagnosis, Real time PCR, tuberculosis.

**تقييم تقنيات Real Time PCR و الكروماتوكرافيا المناعية في التشخيص المختبري للتدرن**

**الخلاصة**

 هذه الدراسة هي دراسة تقييم تشخيصي لطرق (Real time PCR) و الكروماتوكرافيا المناعية (Immunochromatography) مقارنة بالتقنيات المجهرية التقليدية المتمثلة بالصبغة الصامدة للحامض و الزرع في التشخيص المختبري للتدرن. تم خلال الدراسة جمع عينات الدم والبلغم من 136 حالة تدرن 124 حالة تدرن رئوي و 12 حالة تدرن خارج الرئة بالاضافة 102 شخص من الملامسين كمجموعة سيطرة، وذلك في العيادة الاستشارية للامراض الصدرية في الحلة - محافظة بابل خلال الفترة ما بين شهر ايار 2010 الى حزيران 2011. تم استخدام التقنيات التشخيصية للـ (Real time PCR ) و (Immunochromatography) بالاضافة الى الصبغة الصامدة للحامض و الزرع على وسط لونشتن-جنسن في التشخيص المختبري لحالات التدرن. أظهرت نتائج الدراسة ان الحساسية التشخيصية للصبغة الصامدة للحامض، الزرع البكتيري، RT-PCR و الكروماتوكرافيا المناعية كانت 74%، 43% ، 88% و 24% على التوالي. تم الحصول على درجة حساسية تشخيصية بلغت 99% باستخدام كلا تقنيتي الصبغة الصامدة للحامض و RT-PCR. بلغ التخصص التشخيصي 100% لكل من الصبغة الصامدة للحامض و RT-PCR بينما كلن التخصص التشخيصي للكروماتوكرافيا المناعية 98%.

 تبين من النتائج ان استخدام كلا تقنيتي الصبغة الصامدة للحامض و RT-PCR مما ادى الى ارتفاع قيمة الحساسية التشخيصية الى 99% وذلك مقارنة بالحساسية التشخيصية لاستخدام تقنيات الصبغة الصامدة للحامض، الرزع البكتيري، RT-PCR و الكروماتوكرافيا المناعية كفحص تشخيصي منفرد.

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# **Introduction**

T

uberculosis (TB) is an ancient and devastating plague caused by infection with the bacterial pathogen *Mycobacterium tuberculosis*. The disease continues to exact a staggering toll on human life in the present day, killing an estimated 1.7 million people worldwide in the year 2009 alone .

The most widely used rapid test is direct microscopy of a smear of sputum (or other clinical sample) which has been stained for acid-fast bacilli (AFB smear). AFB smear microscopy is the main diagnostic method currently used in most low income countries because it is simple, inexpensive, widely applicable and specific for TB. It is also useful because it has the ability to identify the sickest and most infectious patients. The main disadvantage to smear microscopy is that its overall sensitivity ranges from 20% to 80%, depending on the type of specimen, the patient population, and the technician's performance. Moreover, the sensitivity of smear microscopy is limited in paediatric TB, as well as in HIV-infected patients. Because direct microscopy cannot distinguish between *Mycobacterium tuberculosis* (MTB) and nontuberculous mycobacteria (NTM), specificity is also of concern, although this is not a serious concern in countries with high TB incidence. AFB smear is also labour intensive, so in countries with high cost of labour this will not be cheap .

Mycobacterial culture is more sensitive and specific for the diagnosis of TB than AFB smear and is considered the gold standard in diagnostics for TB. The main disadvantage of mycobacterial culture is the long incubation period to get the results .

Nucleic acid amplification tests amplify target sequences of DNA or RNA from the MTB organisms. These tests are complex and expensive but have advantages, including high specificity, greater sensitivity than smear microscopy, with an average of about 80% compared to culture. They are also very quick as they can provide results within one day. However, sensitivity is modest in smear-negative pulmonary TB, and in extra-pulmonary disease averages 50-60%. False positives can also be very frequent, because without meticulous laboratory procedures and techniques, cross-contamination is frequent, which can lead to all specimens being positive ‎[4]. Real-time PCR offers several advantages over conventional PCRs: (i) it provides quantitative data; (ii) it reduces the risk of cross-contamination by minimizing the need for post-PCR handling; (iii) hands-on time is reduced; (iv) turnaround time is reduced; and (v) it offers high sensitivity and specificity .

Serodiagnostic tests using various antigens to measure antibodies to *M. tuberculosis* in serum offer several advantages. Serodiagnosis is potentially useful for early diagnosis of both pulmonary and extrapulmonary TB, even before clinical manifestation of disease. It does not require collection of specimens from the site of disease and is, therefore, especially feasible for TB diagnosis in patients with extrapulmonary disease and in young children who are usually incapable of providing sputum for AFB smear and culture examination ‎[6].

This study aimed to evaluate the real-time PCR (RT-PCR) and commercial immunocromatogaphic technique as laboratory diagnostic techniques to tuberculosis compared to conventional (Acid fast bacilli smear and culture).

# **Materials and Methods**

**Patients and Control Subjects**

Study population included patients with suspected pulmonary or extrapulmonary tuberculosis from Babil province - Iraq. Whole blood and sputum samples were collected from 136 TB cases at consultant clinic for respiratory diseases in Hilla – Babil province in the period between May 2010 to June 2011. Laboratory criteria for diagnosis were include the Isolation of *M. tuberculosis* from a clinical specimen or Demonstration of *M. tuberculosis* complex from a clinical specimen by nucleic acid amplification test or Demonstration of acid-fast bacilli in a clinical specimen when a culture has not been or cannot be obtained or is falsely negative or contaminated. The inclusion criteria used in this study was that all the TB cases must meet the clinical diagnosis criteria and confirmed by at least one of the laboratory diagnosis tests (Smear, PCR and culture). The clinical diagnosis were conducted by the specialists in the consultant clinic for respiratory diseases.

Out of the 136 TB patients, there were 76 male and 60 female. The patients age range was from 12 to 80 years (the mean 38.7 years , median 34.5 years). Of the 124 TB patients, 100 (80.6%) were attended as a new TB patients. The remaining 24 (19.4%) patients were attended to follow-up evaluation of the DOT (Directly observed treatment) program. All extrapulmonary patients were attended as new TB cases. The extrapulmonary TB cases were diagnosed by AFB smear of tissue biopsy.

The control specimens were collected from 102 healthy subjects. From those subjects there were 35 health care workers who works at the subjects from consultant clinic for respiratory diseases in Hilla city – Babylon , Iraq. The rest 67 control samples were obtained from non-relative healthy contacts (patients non-relative spouses). The control samples included 128 whole blood samples in addition to 102 sputum samples. 26 control subjects didn't provide a sputum sample and so they excluded from the study.

**Samples Preparation**

Two sputum specimens in two days (one sample each day) were obtained from all suspected TB patients. The sputum was collected early morning before the patient has eaten. In addition to that one sputum specimen was obtained from each control subject. All sputum samples were processed by the standard N-acetyl-L-cysteine–sodium hydroxide (NACL-NaOH) method ‎[8].

Bacteriological Diagnosis

The smear for acid fast stain was prepared according to WHO guidelines ‎[9]. A minimum of 100 fields were examined before the smear is reported as negative. Acid-fast bacilli are approximately 1-10μm long and typically appear as slender, rod-shaped bacilli, but they also may appear curved or bent ‎[9]. The recording and reporting of the results was according to the WHO standards .

After initial decontamination / digestion by NACL-NaOH method, all sputum samples were cultured on solid Lowenstein-Jensen (LJ) media and liquid (Dubos broth) medium. The LJ medium that used in this study was provided by HiMedia Laboratories, India. In order to reduce contamination of LJ slants with respiratory normal flora, the Gruft Mycobacterial Supplement (supplied by HiMedia Laboratories) was added to LJ slants. This antibiotic and enrichment supplement is recommended for selective cultivation of Mycobacteria .

The LJ slants and Dubose broth were incubated in 35-37ºC for 10-12 weeks. The cultures were examined weekly for bacterial growth (World Health Organization. 1996). Members of the *M. tuberculosis* complex do not grow within three days at 37ºC. Acid fast stain slides were prepared from positive cultures and the positive acid fast stain cultures were considered as Mycobacterium at genus level ‎[10]. *M. tuberculosis* don't produce any pigment even after exposure to light. *M. tuberculosis* light buff color rough colonies while some other members of Mycobacterium species is capable of producing pigments like yellow or orange on solid media . The niacin test, pyrazinamidase test and nitrate reduction test were performed for all positive cultures in order to identify Mycobacterium tuberculosis at species level.

Serological Diagnosis of Tuberculosis by Immunochromatography (ICG) Assay.

The anti-TB antibodies (IgG, IgM, IgA) in serum samples of both PTB cases and controls were detected by using Tuberculosis Rapid Test Device provided by Acon, USA. The ICG assay uses a double antigens “sandwich principle” for the detection of Tuberculosis antibody in human whole blood, serum or plasma. Two recombinant Tuberculosis antigens (TB Ag 1and 2) were mixed and immobilized on the test band region, and an antibody to M. tuberculosis on the control band region of nitrocellulose membrane. Another Tuberculosis antigen (TB Ag 3), coupled with colloidal gold particles, is dried on a conjugate pad. During the assay, the specimen is allowed to react with the colored conjugate (antigen-colloid gold conjugate); the mixture then migrates chromatographically along the membrane by capillary action. If the specimen contains anti- *M. tuberculosis* antibody, the recombinant antigen immobilized on the membrane will capture the antibody-antigen-colloidal gold complex and form a colored test band on the membrane, indicating a positive result. Absence of the test band suggests a negative result. To serve as a procedural control, a colored band at control region always appears in the test area.

Direct Molecular Detection and Identification of Mycobacteria

The mycobacterial DNA was extracted from sputum samples by using DNA extraction kit (DNA-Sorb-B DNA extraction kit) provided by Sacace Biotechnologies-Italy. All sputum samples were digested and decontaminated by the standard NACL-NaOH method. The mycobacterial DNA was extracted according to the manufacturer instructions.

The real-time PCR (RT-PCR) technique was used in this study for direct molecular diagnosis of tuberculosis infection in subjects included in this study. AccuPower® MTB&NTM Real-Time PCR Kit provided by (Bioneer Co.,Korea) was used for RT-PCR based M. tuberculosis diagnosis. The AccuPower® MTB&NTM Real-Time PCR Kit is composed of a ready-to-use format for real-time qualitative detection of Mycobacterium tuberculosis (MTB) and Non-tuberculous mycobacteria (NTM) DNA in clinical samples. PCR thermal cycling conditions (40 cycles of; 10 minutes at 95℃, 20 second at 95 ℃ and 30 second at 55℃) were carried out in Exicycler™ 96 Real-Time Quantitative Thermal Block (Bioneer Co., KOREA).

Calculation of Sensitivity, Specificity and Accuracy

To calculate diagnostic sensitivity and specificity the following formulas are used ‎[11]:




# **Results**

**Afb Smear Diagnosis Of Pulmonary Tb Patients**

A total of 136 TB patients were enrolled in this study that meet the clinical diagnosis criteria and were positive for at least 1 of the laboratory diagnosis criteria. Among those patients, there were 124 pulmonary TB (PTB) cases, in addition to 12 subjects with extrapulmonary TB cases. Of the extrapulmonary cases, there were 10 cases of TB lymphadenitis and 2 cases of bone TB.

As indicated in "Table 1" was shown that of the 124 PTB cases, there were 92 (74%) AFB smear positive cases, 54 (43%) culture positive, 110 (88%) RT-PCR positive and only 30 (24%) patients were positive for immunochromatographic assay (ICG). All the extrapulmonary cases were AFB smear positive in tissue biopsy not in sputum specimen. Six of the extrapulmonary TB cases were positive for ICG "Table 1".

Out of the 124 PTB cases, There were 92 (74%) AFB smear positive cases. "Table 2" was shown AFB smear results of the PTB patients compared with results of the other techniques. Of the 92 positive AFB smears, there were 80 (87%) positive RT-PCR results, 42 (46%) positive culture results and 24 (26%) positive ICG results.

**Culture Diagnosis Of Pulmonary Tb Patients**

Out of the 124 PTB cases, There were 54 (43%) culture positive cases. "Table 3" was shown culture results of the PTB patients compared with results of the other techniques. Of the 54 culture positive cases, there were 48 (89%) positive RT-PCR results, 42 (78%) positive AFB smear results and 22 (41%) positive ICG results.

**Real-Time Pcr Diagnosis Of Pulmonary Tb Patients**

Out of the 124 PTB cases, There were 110 (88%) RT-PCR positive cases. "Table 4" was shown RT-PCR results of the PTB patients compared with results of the other techniques. Of the 110 RT-PCR positive cases, there were 80 (73%) positive AFB smear cases48 (44%) culture positive cases and 24 (22%) positive ICG cases.

**Immunochromatography (Icg) Diagnosis Of Pulmonary Tb Patients**

Out of the 124 PTB cases, There were 30 (24%) ICG positive PTB cases. "Table 5" was shown ICG results of the PTB patients compared with results of the other techniques. Of the 30 ICG positive cases, there were 24 (80%) RT-PCR positive cases, 24 (80%) AFB smear positive cases and 11 (37%) culture positive cases.

Out of the 12 extra-pulmonary TB cases, There were 6 (50%) ICG positive PTB cases "Table 1". The sputum samples of those extra-pulmonary TB cases were negative for AFB smear, RT-PCR and culture.

**Sensitivity, Specificity And Accuracy Of The Laboratory Diagnosis Techniques**

Regarding to the confirmed 124 PTB cases in this study, the sensitivity of the AFB smear, culture, RT-PCR and ICG were 74%, 43% 88% and 24%, respectively. The specificity of the AFB, Culture and RT-PCR were 100% whereas the specificity of ICG assay give 98% due to 2 false positive results among the 102 control subjects. Therefore the accuracy (93%) were obtained from RT-PCR results followed by AFB smear (85%) as shown in "Table 6". By using both AFB smear and RT-PCR as diagnostic tests raises the sensitivity up to 99% as compared with the using of the AFB smear, culture and ICG as a single test (fig:1).

When the culture was considered the gold standard (only culture positive cases included), the AFB smear, RT-PCR and ICG sensitivities were 77.8%, 92.6% and 40.7%, respectively. On the other hand, the RT-PCR exhibited accuracy (97%) as compared with AFB smear ( 92%) and the ICG (78%) (table 7).

# **Discussion**

A variety of NAA-based assays has been commercially developed for detection of MTBC and NTM from clinical specimens and are now widely used in clinical microbiology laboratories. In particular, the use of real-time PCR assay for the detection of microorganisms has been increasing, replacing conventional PCR that uses agarose gel electrophoresis for identification of PCR products. This assays have the advantage of a low contamination risk and simultaneous identification of multiple targets .

The better sensitivity reported in this study was the RT-PCR 88% in confirmed PTB cases and 92.6% in culture positive PTB cases, the specificity was 100% (tables 6&7). These results are with an accordance with those results being reported by Inoue *et al.* was stated that the real-time PCR assay with an internal control achieved a sensitivity of 84% and specificity of 97% ‎[13].

The PCR based method of MTBC enables detection of as few as 1 to 10 bacilli in clinical specimen such as sputum and other body fluids. Relative to smear, which requires a relatively large bacilli load (104/mL) and also identifies other acid-fast bacteria, the approach has significantly enhance the sensitivity and specificity of laboratory diagnosis . In addition, the tests can be performed within a few hours, making them attractive against culture for rapid diagnosis.

It has been reported that the sensitivity and specificity of PCR ranges from 50 to 100%. It has been suggested that this wide range may be due to the variability of PCR results in different laboratories ‎[16].

In laboratory trials of the FDA-approved TB diagnostic PCR assays, sensitivity in respiratory specimen has ranged from 50% to 95%, and specificity has ranged from 95% to 100% ‎[2]. A meta-analysis combining all formats (n=125 studies) estimated overall sensitivity of 85% and specificity of 97% . PCR specificity is consistently very high. Sensitivity is consistently highest in AFB smear-positive specimens, and lowest in specimens from smear-negative ‎[18].

Another meta-analysis of over 60 studies showed that pooled sensitivity and specificity were respectively 96% and 85% in smear-positive and 66% and 98% in smear negative respiratory samples ‎[19].

This study showed that of the RT-PCR were positive in 93.7% of smear negative PTB cases. The result was higher than that reported by other study of the performance of PCR tests for the diagnosis of tuberculosis which reported that sensitivity was 66-73% in smear-negative samples .

Nicol and Zar were reported that a sensitivity of 66% for smear-negative TB by using different commercial PCR assays .

Rapid identification of MTBC in smear-negative samples as well as in smear-positive samples is important for prevention of tuberculosis transmission, because about 17% of tuberculosis cases involve transmission from persons with negative AFB smear results [.

On the other hand, The results of AFB smear in this study showed that a sensitivity of 74% and 77.8% in confirmed PTB and culture positive PTB cases. The specificity of AFB smear was 100%. Direct microscopic examination of sputum for AFB was inexpensive, rapid, and relatively easy to perform. Compared to mycobacterial culture, the sensitivity of a single sputum AFB smear is 30% to 40%, but it increased to reach 65% to 80% with multiple specimens or concentrated sputum . The AFB smear mainly has two disadvantages. First, The sensitivity of AFB smear is such that it requires 6000 to 10,000 organisms per mL of sample to register as positive case. The second disadvantage is direct microscopy cannot distinguish between M. tuberculosis and nontuberculous mycobacteria (NTM) .

In this study, TB culture showed sensitivity of 43% and specificity of 100%. The low sensitivity of TB culture in this study can be attributed to the anti-TB antibiotic administration by many PTB patients included in this study.

It has been mentioned that by using of final clinical diagnosis of pulmonary TB as the standard, the sensitivity of sputum culture is greater than 80%. Mainly because of laboratory contamination of the specimen at the bedside or in the laboratory, false positives also occur with culture. Nevertheless, the specificity of culture has been reported to be as high as 98% .

Culture requires an average of 21days to obtain results; even the Bactec radiometric technique (Becton Dickinson, USA) requires 13 days to produce results. Whoever, the sensitivity of culture ranges from 50% to 81% .

The combination of two tests, with a positive result by either of the tests, increases the diagnostic sensitivity for all individual tests . Alsmeda *et al.* were reported that the combination of PCR and stain, both of which are faster than culture, has a sensitivity similar to that of culture . The ICG assay exhibited a poor sensitivity (24%) in confirmed PTB cases and 40.7% in culture confirmed PTB cases (tables 6 & 7). The sensitivity of ICG was better in extrapulmonary TB cases (50%) as compared with its sensitivity in PTB.

Some studies have compared serodiagnostic tests in known cases of TB against healthy controls. Their use has been disappointing in clinical practice where true TB cases must be distinguished from patients suspected of having TB but who in fact have other conditions. The kits differ in features including test format (i.e., modifications of ELISA or immunochromatographic tests), antigen composition (e.g., secreted and heat shock proteins, lipopolysaccharides, or peptides), and class of detected immunoglobulins (e.g., IgA, IgG, IgM) ‎[27]; ‎[28].

# **Conclusions**

The sensitivity of RT-PCR is higher compared to acid fast staining, culture and serology. The using of both AFB smear and RT-PCR as diagnostic tests for tuberculosis showed the higher sensitivity as compared with the using of the RT-PCR, AFB smear, culture and ICG as a single test. For a rapid, more sensitive and specific laboratory diagnosis of tuberculosis, the use of both AFB smear and RT-PCR is recommended.

**Table 1** Resultsof laboratory tests of the TB patients in this study:

|  |  |
| --- | --- |
| Laboratory Diagnostic test | No. of positive patients (%)  |
| PTB (n=124) | Extrapulmonary TB (n=12) |
| AFB Smear | 92 (74%) | 12 (100%) |
| Culture | 54 (43%) | 0 |
| RT-PCR | 110 (88%) | 0 |
| ICG  | 30 (24%) | 6 (50%)  |

**Table 2** AFB smearresults of 124 specimens of PTB patients compared with RT-PCR, culture and ICG:

|  |  |  |  |
| --- | --- | --- | --- |
| Results | RT-PCR | Culture | ICG |
| +ev | -ev | +ev | -ev | +ev | -ev |
| AFB Smear | +ev 92 | 80 | 12 | 42 | 50 | 24 | 68 |
| -ev 32 | 30 | 2 | 12 | 20 | 6 | 26 |
| Total 124 | 110 | 14 | 54 | 70 | 30 | 94 |

**Table 3** Culture results of 124 specimens of PTB patients compared with RT-PCR, AFB smear and ICG:

|  |  |  |  |
| --- | --- | --- | --- |
| Results | RT-PCR | AFB Smear | ICG |
| +ev | -ev | +ev | -ev | +ev | -ev |
| Culture | +ev 54 | 48 | 6 | 42 | 12 | 22 | 32 |
| -ev 70 | 62 | 8 | 50 | 20 | 8 | 62 |
| Total 124 | 110 | 14 | 92 | 32 | 30 | 94 |

**Table 4** RT**-** PCR results of 124 specimens of PTB patients compared with AFB smear, culture and ICG:

|  |  |  |  |
| --- | --- | --- | --- |
| Results | AFB Smear | Culture | ICG |
| +ev | -ev | +ev | -ev | +ev | -ev |
| RT-PCR | +ev 110 | 80 | 30 | 48 | 62 | 24 | 86 |
| -ev 14 | 12 | 2 | 6 | 8 | 6 | 8 |
| Total 124 | 92 | 32 | 54 | 70 | 30 | 94 |

**Table 5** ICG results of 124 specimens of PTB patients compared with AFB smear, culture and RT**-** PCR:

|  |  |  |  |
| --- | --- | --- | --- |
| Results | RT-PCR | AFB Smear | Culture |
| +ev | -ev | +ev | -ev | +ev | -ev |
| ICG | +ev 30 | 24 | 6 | 24 | 6 | 11 | 19 |
| -ev 94 | 86 | 8 | 68 | 26 | 43 | 51 |
| Total 124 | 110 | 14 | 92 | 32 | 54 | 70 |

**Table 6** Sensitivity, specificity and accuracy of AFB smear, culture, RT-PCR and serology tests analyzed in 124 PTB patients and 102 control subjects:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Test | No. of subjects  | Sensitivity | Specificity | Accuracy  |
| **True positive** | **False negative** | **True negative** | **False positive** |
| AFB smear | 92 | 32 | 102 | 0 | 74% | 100% | 85% |
| Culture | 54 | 70 | 102 | 0 | 43% | 100% | 69% |
| RT-PCR | 110 | 14 | 102 | 0 | 88% | 100% | 93% |
| ICG | 30 | 94 | 100 | 2 | 24% | 98% | 57% |

**Table 7** Sensitivity, specificity and accuracy of AFB,RT-PCR and serology tests analyzed in 54 culture positive PTB patients and 102 control subjects:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Test | No. of subjects  | Sensitivity | Specificity | Accuracy  |
| **True positive** | **False negative** | **True negative** | **False positive** |
| AFB Smear | 42 | 12 | 102 | 0 | 77.8% | 100% | 92% |
| RT-PCR | 50 | 4 | 102 | 0 | 92.6% | 100% | 97% |
| ICG | 22 | 32 | 100 | 2 | 40.7% | 98% | 78% |

**Figure 1** Comparison between the sensitivity of the each single TB diagnostic test and combination of RT-PCR and AFS smear.

# **53.jpg**

**Figure 2** Results of Real time-PCR. The dark curve represent the florescence of MTB- specific probe (FAM labeled probe) which indicate a positive PCR amplification of M tuberculosis. The light curve represent the internal positive control (TAMRA labeled probe).

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