One day symposium on TB field diagnostics

TB DYING FOR A TEST

Abstracts

CAMPAIGN FOR ACCESS TO ESSENTIAL MEDICINES

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CAPE TOWN 7 NOVEMBER 2007

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Session 1: Introduction & Current Field Diagnostics

Time	Session	Presenter
8.30	Registration, poster mounting, coffee	
8.55	Welcome and introduction	Tido von Schon-Angerer, MSF
9.00- 10.35 95mins	Session 1: TB field diagnostics	Chairs: Nathan Ford, MSF
9.00-9.10	Optimization of sputum sample quality	Mishal Khan, Pakistan/LSHTM
9.10-9.25	Microscopy: Something Old, Something New...	Kevin Fennelly, New Jersey Medical School, New Jersey, USA
9.25-9.45	Decontamination & culture : best techniques for different settings	Carlton Evans, Peru/IFHAD
9.45-9.55	Prize talk 1: Is there still a place for conventional methods in the rapid detection of rifampin and isoniazid resistance in M. tuberculosis? The case of thin layer agar direct method	Jaime Robeldo, Colombia
9.55-10.05	Prize talk 2: Optimisation Of Tb Field Testing: In-Transit Sputum Decontamination & Culture On Colorimetric Selective Media For Tb Diagnosis & Drug-Susceptibility Testing	Beatriz Herrera, Peru/IFHAD
10.05-10.35	Questions & Discussion	Panel of all of above
10.35-11.00 25mins	Coffee and poster viewing	

Session 2: Operational Challenges for Field Diagnostics

11.00-12.40 100mins	Session 2: Challenges of field diagnosis	Chairs: Francis Varaine Paul Van Helden, Stellenbosch
11.00-11.20	TB in HIV positive people: Ruling out TB & diagnosing TB & MDRTB	Helen Ayles, Zambia/London
11.20-11.35	The challenge of diagnosing paediatric TB	Heather Zar, UCT
11.35-11.50	Diagnostic biosafety : What's required for what tests?	Paul Jensen, CDC
11.50-12.10	Prize talk: "Estimating the resource need for using culture to diagnose TB"	Dirk Mueller, LSHTM
12.10-12.40	Questions & Discussion	Panel of all of the above
12.40-2.00	Lunch and poster viewing	

Session 3: Future Field Diagnostics

2.00- 3.00	Session 3: Current pipeline and future tests	Eric Goemaere, MSF Ruth McNerney, LSHTM
2.00-2.10	Field tests: What, where & why?	Gilles van Cutsem, MSF S Africa
2.10-2.25	Current pipeline for commercial diagnostic tests	Mark Perkins, FIND
2.25-2.40	Current pipeline for non-commercial diagnostic techniques	Andrew Ramsay, TDR/WHO
2.40-3.10	Discussion: will the current pipeline produce what patients need?	Panel including all of the above + Anandi Martin, IMT Antwerp + Pamela Hepple, Manson Unit MSF
3.10-3.40 30mins	Coffee and posters	

Session 4: Encouraging Innovation & Progress in Field Diagnostics

3.40- 5.20	Session 4: How to make innovation happen	Chairs Mark Harrington, TAG Marc Mendelson, UCT
3.40- 3.50	What are the research gaps to be filled?	Rob Wilkinson, UCT/Imperial
3.50- 4.00	Why aren't best tests being used?	Dave Moore, Peru/Imperial
4.00- 4.10	Is there enough money?	Javid Sayed, TAG
4.10- 4.20	Why are TB diagnostics still so inadequate?	Martine Usdin, MSF
4.20 -5.20	Discussion & Questions	Panel including all of above, plus: Val Snewin, The Wellcome Trust Denny Mitchison, St George's, London
5.20-5.25	Poster prize for best abstract presented as a poster	
5.25-5.40	Conclusions	Tido von Schon-Angerer, MSF Carlton Evans, Peru/IFHAD
5.40-7.00	Reception	

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Session 3: Future Field Diagnostics

Clinical, Immunological and Epidemiological Importance of Anti-Tuberculosis T Cell Responses in HIV Infected Africans

M.X. Rangaka[1], L. Diwakari, R. Seldoni, G. van Cutsem[2],4 , G. A Meintjesi,[3], C. Morroni [4], P. Mouton [1,3], M. S. Sheyi, G. Maartens[1,5,6] , K.A. Wilkinson[1,7], R. J. Wilkinson[1,7]

Background:

HIV-associated tuberculosis is a major cause of mortality in Africa. The assay of T-cell interferon-gamma released in response to antigens of greater specificity than PPD is a useful improvement over the Mantoux skin test, but few studies have evaluated Interferon-gamma secretion in HIV-infected people. Were it to be convincingly shown that *in vitro* tests of sensitisation were less impaired by HIV, this might justify their wider implementation in resource-poor environments, despite their inherently greater complexity and expense.

Study Aims:

Our first aim was to determine differences in *M. tuberculosis* (MTB) antigen recognition between HIV-infected and uninfected people living in the same area. Our second aim was to determine whether differences exist between HIV-infected people with and without active pulmonary tuberculosis. Thirdly, we investigated whether differences in the pattern of antigen recognition between groups correlated with TB disease status, in order to gain insight into the potential diagnostic utility of these responses.

Setting & Methods:

Recruitment was conducted at the Ubuntu HIV-TB clinic in the township of Khayelitsha, South Africa. There is an extremely high burden of both tuberculosis (TB) and HIV infection with an annual TB incidence of 1,612/100,000 and an antenatal HIV seroprevalence of 33% in 2005. HIV seropositive persons with newly-diagnosed pulmonary tuberculosis (HIV+PTB) were enrolled within five doses of starting anti-tuberculosis chemotherapy: all were culture or sputum smear positive for *M. tuberculosis.* Persons newly diagnosed as HIV seropositive but without signs or symptoms of active TB (HIV+ subjects), and healthy persons found to be HIV seronegative (HIV- subjects) were enrolled sequentially shortly after attending the same voluntary counselling and testing (VCT) clinic for HIV. Both HIV+ and HIV- subjects were screened for active tuberculosis by a symptom-score questionnaire. Current antibiotic, antiviral and steroid therapy were exclusions, as were pregnancy, age < 18 and any acute illness other than TB. HIV+ persons qualifying for antiretroviral therapy under current guidelines (CD4 count < 200 /mm^3 or WHO clinical stage 4) were referred to the antiretroviral clinic. *M. tuberculosis* antigen specific IFN-gamma secretion was assessed by whole blood and ELISpot methods and compared to the Mantoux skin test in HIV-infected (HIV+) and uninfected (HIV-) people without active tuberculosis; and HIV-infected patients with pulmonary tuberculosis (HIV+PTB) in Khayelitsha, South Africa.

Results:

By comparison with HIV- subjects, the skin test and whole blood responses to PPD in HIV+ subjects were depressed ($p<0.001$). By contrast, the responses to *M. tuberculosis* antigens (ESAT-6, CFP-10, TB10.3 and Acr2) were less affected, indicating a high prevalence of latent tuberculosis ($\sim$ 80%) in both HIV- and HIV+ subject groups. Whole blood responses did not differ between the HIV+ subjects and HIV+PTB groups, but the ELISpot response to ESAT-6 and CFP-10 was higher in the HIV+PTB ($p \leq 0.04$), although this group had lower CD4 cell counts. A ratio of the combined ELISpot response divided by the CD4 count of > 1.0 had 88% sensitivity and 80% specificity for active pulmonary tuberculosis in HIV infected people.

Conclusions:

Interferon-gamma release appears less impaired by HIV co-infection than skin testing. The novel potential to relate the ELISpot and CD4 counts to assist diagnosis of active tuberculosis in HIV is important, and deserves further evaluation.

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Effect of HIV-1 Infection on T-Cell–Based and Skin Test Detection of Tuberculosis Infection

M. X. Rangaka[1,2], K. A. Wilkinson[1,2], R. Seldon[1], G. van Cutsem[3], G. A. Meintjes[1], C. Morroni[4], P. Mouton[1], L. Diwakar[1], T.G. Connell[1], G. Maartens[1,5], and R. J. Wilkinson[1,2,6]

Rationale: Two forms of the IFN-γ release assay (IFNGRA) to detect tuberculosis infection are available, but neither has been evaluated in comparable HIV-infected and uninfected persons in a high tuberculosis incidence environment.

Objective: To compare the ability of the T-SPOT.TB (Oxford Immunotec, Abingdon, UK), QuantiFERON-TB Gold (Cellestis, Melbourne, Australia), and Mantoux tests to identify latent tuberculosis in HIV-infected and uninfected persons.

Setting & Methods: A cross-sectional study of 160 healthy adults without active tuberculosis attending a voluntary counselling and testing centre for HIV infection in Khayelitsha, a deprived peri-urban South African community with an HIV antenatal seroprevalence of 33% and a tuberculosis incidence of 1,612 per 100,000. Adults resident in Khayelitsha and attending voluntary testing and counselling for HIV infection were invited to participate. Persons testing seronegative for HIV-1 were invited to participate after voluntary testing and counselling, on the day of their negative test. Recruitment of persons found to be HIV seropositive was deferred until their first visit to the HIV/AIDS clinic, because we did not wish to recruit them before they had had time to accept their HIV diagnosis. Prior Tuberculosis, IPT and steroid therapy were exclusions, as were pregnancy and (in HIV-infected people) current opportunistic infection or a Karnofsky score not exceeding 60. Assessment of eligibility included a symptom-screening questionnaire and physical examination for active tuberculosis based on validated approaches. The presence of any one of the following – cough, chest pain, recent weight loss, night sweats, fever, loss of appetite, swelling of lymph nodes, or generalised tiredness – formed an exclusion criterion and triggered referral.

At the first study visit blood was taken and the intradermal (Mantoux) skin test was performed by placing tuberculin PPD RT23 2TU on the volar aspect of the forearm. On the second study visit, the transverse diameter of the TST induration was determined by the ballpoint pen and ruler method. HIV-infected persons with Mantoux reactions of or exceeding 5 mm were offered IPT according to South African national guidelines. Blood samples were processed within four hours of drawing. The T-SPOT.TB and QFT-G assays were performed and interpreted according to the manufacturer's insert guidelines (T-SPOT.TB). Laboratory staff members were blind to the clinical status of samples. Scoring was carried out by a third investigator who had no role in clinical recruitment or in performing the laboratory assays. In HIV-infected persons, the CD4+ cell count was simultaneously determined: people qualifying for antiretroviral therapy (CD4+cell count less than 200/mm3 or World Health Organisation clinical stage 4) were referred to the antiretroviral clinic.

Measurements & Main Results: One hundred and sixty (74 HIV+ and 86 HIV–) persons were enrolled. A lower proportion of Mantoux results was positive in HIV-infected subjects compared with HIV uninfected subjects ($p < 0.01$). By contrast, the proportion of positive IFNGRAs was not significantly different in HIV-infected persons for the T-SPOT.TB test (52 vs. 59%; $p = 0.41$) or the QuantiFERON-TB Gold test (43 and 46%; $p = 0.89$). Fair agreement between the Mantoux test (5- and 10-mm cutoffs) and the IFNGRA was seen in HIV-infected people ($\kappa = 0.52–0.6$). By contrast, poor agreement between the Mantoux and QuantiFERON-TB Gold tests was observed in the HIV-uninfected group ($\kappa = 0.07–0.30$, depending on the Mantoux cutoff). The pattern was similar for T-SPOT.TB ($\kappa = 0.18–0.24$).

Summary: IFNGRA sensitivity appears relatively unimpaired by moderately advanced HIV infection. However, agreement between the tests and with the Mantoux test varied from poor to fair. This highlights the need for prospective studies to determine which test may predict the subsequent risk of tuberculosis.

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Queues at a rural clinic in Lesotho

TB DYING FOR A TEST

Session 1

Current Field Diagnostics

FIND Demonstration Projects: MGIT Culture and Drug Susceptibility Testing

Heather Alexander[1,2], Hojoon Sohn[2], Richard O'Brien[2]

In December 2004, the Foundation for Innovative New Diagnostics (FIND) and Becton Dickenson and Company (BD) signed a formal agreement under which FIND would perform Mycobacterial Growth Indicator Tube (MGIT) culture and drug susceptibility testing (DST) demonstration projects, with BD providing MGIT equipment and reagents at affordable and sustainable rates to the public sector in high-burden TB countries through 2009. In 2005, in consultation with the Green-Light Committee (GLC) and WHO, FIND selected four demonstration project sites in support of MDR TB treatment programmes: Nukus, Uzbekistan (in collaboration with MSF), Kathmandu, Nepal (in collaboration with GENETUP), Manila, Republic of the Philippines (in collaboration with the Tropical Disease Foundation), and Samara Oblast, Russian Federation (in collaboration with the University of London and UK Health Protection Agency Mycobacterium Reference Laboratory).

Each MGIT demonstration project is in a different stage of implementation and patient outcomes are not yet available. To date, laboratory performance data have been received for a total of 1831 sputum specimens with smear microscopy, MGIT culture, and LJ culture results, collected from patients at high risk of MDR TB. The MGIT positivity rate was 55% (1012/1831), compared to 41% (744/1831) culture positivity on LJ MGIT and LJ recovery rates (MGIT culture positive/culture positive by any method; LJ culture positive/ culture positive by any method) were similar for smear positive specimens (90% MGIT, 87% LJ). However, the MGIT recovery rate for smear negative specimens was 93%, compared to only 41% on LJ. Median time to detection of positive cultures was approximately 19 days faster in MGIT (9 days, IQR 6-15) than on LJ (28 days, IQR 21-36). A preliminary analysis of limited DST data indicates that the rate of agreement between LJ DST and MGIT DST results for isoniazid and rifampicin is 99% and that for isoniazid, rifampicin, streptomycin, and ethambutol is 89%.

Detailed cost estimates using FIND preferential pricing for MGIT and local costs from the Samara MGIT DST project has been compiled using a laboratory cost estimation tool (LCET), designed for this purpose. Case finding costs for LJ and MGIT were approximately $9 and $10, respectively. Testing costs were approximately $18 for MGIT compared to $14 with LJ for isoniazid and rifampin DST in patients at high risk of MDR TB.

The implementation of liquid culture and DST in these four programmatic settings has been met with numerous challenges, some of which have required innovative solutions. Automated testing with the MGIT 960 instrument in settings where the power supply is frequently interrupted requires additional back-up power to maintain the incubation temperature and ensure that data are not lost from the computer. The technological complexity, bio-safety and contamination risk associated with liquid culture and DST systems significantly increases the training demands. Additional programme challenges include delays in instrument and consumables ordering, shipment, customs clearance and delivery. However, none of these challenges is insurmountable, and issues can be resolved with careful planning.

The FIND demonstration projects have shown that although challenging, implementation of liquid culture and DST methods in low-resource settings is feasible. At the FIND preferential prices, testing costs for culture and DST by MGIT are only slightly higher than for LJ and should be weighed against the potential patient benefits derived from the increased culture sensitivity and decreased time to detection afforded by the use of MGIT. In June 2007, FIND presented data from these projects to the WHO Strategic and Technical Advisory Group for TB, which subsequently recommended endorsement of liquid culture and DST in low and medium income settings.

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Can the Line Probe Assay inno-lipa rif.tb be Used for Detection of MDR-TB in Low-resource Countries? Results of an Implementation Validation in Rwanda

Cindy Maria Quezada[1], Eliane Kamanzi[2], Julienne Mukamutara[3], Pim de Rijk[4], Leen Rigouts[4], Françoise Portaels[4], Yanis Ben Amor[5*]

Background: Along with HIV/AIDS, multidrug-resistant tuberculosis (MDR-TB) has become the most important threat to TB control. A recent survey performed in Rwanda according to WHO guidelines determined the combined prevalence of MDR-TB to be 4.6%, 1.7 times higher than that reported for Africa (2.7%). Left untreated or inappropriately treated, MDR-TB could become an emerging threat in Rwanda and other African countries displaying similar trends in drug resistance. Implementing appropriate management policies is therefore crucial, especially in light of the emergence of extensively drug-resistant TB (XDR-TB).

Objectives: Late recognition of drug resistance contributes considerably to the mortality of patients and the spread of MDR-TB and XDR-TB, particularly among immune-compromised patients. The timely detection of drug-resistant TB is therefore imperative. In this study, we validated in a resource-poor setting INNO-LiPA Rif.TB, a diagnostic test based on the Line Probe Assay platform for rapid detection of MDR-TB and XDR-TB.

Methods: Blinded samples, a mix of susceptible and rifampicin resistant isolates from tuberculosis patients, were analysed using INNO-LiPA Rif.TB in Rwanda, testing three working conditions: the facility, the resources used and the skills of the operator. In each setting the samples were analysed in parallel by a Rwandan technician and a trained molecular biologist, whose results served as a control. To determine whether INNO-LiPA could be carried out in Rwanda using local resources, we tested two key materials required for the PCR step but not provided in the kit: Taq polymerase and water: we analysed each sample, in each setting, using either PCR-grade distilled water and Taq polymerase purchased from the US, or locally distilled water and a cheaper Taq polymerase purchased from Kenya, the nearest geographical distributor of the enzyme. Successful validation of the test in Rwanda was assessed by comparing results obtained for the same samples at the Institute of Tropical Medicine (Antwerp) in Belgium.

Findings: No substantial difference was found between INNO-LiPA Rif.TB results obtained in Rwanda and Belgium with the same samples. The tool was also effectively implemented using cheaper local Taq polymerase and distilled water. Technicians trained for the technique were not molecular biologists as the technology was simple enough to transfer.

Conclusions: The rapid diagnostic test for MDR-TB and XDR-TB can therefore be reliably implemented in a resource-poor setting such as Rwanda.

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Evaluation of Microscopic-Observation Drug-Susceptibility (MODS) vs. Clinical Assessment, Sputum Microscopy, Culture and PCR for Diagnosing Patients with Tuberculosis in a Resource-poor Setting

Oliver C. Boney[1], Karine Zevallos[1,2], Robert H. Gilman[1,2,3], Luz Caviedes[1,2], Teresa Valencia[2], Carlos O. Vidal[4], A. Roderick Escombe[5], Carlton A. Evans[1,2,3,5]

Background: Diagnostic difficulties hamper tuberculosis control.

Objective: To compare the performance of strategies for diagnosing tuberculosis.

Design: A prospective comparison of symptomatic assessment, two microscopy techniques (Ziehl-Neelsen and auramine), PCR and three culture-based techniques: Microscopic Observation Drug Susceptibility technique (MODS), Lowenstein-Jensen (LJ) and the indirect Microplate Alamar Blue Assay (MABA).

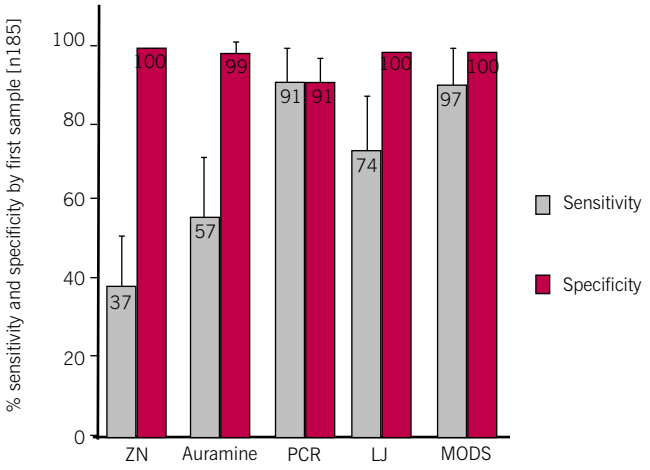
Subjects: 185 patients with symptoms suggestive of tuberculosis.

Setting: A hospital clinic in the Peruvian Amazon and a university pathology laboratory.

Methods: 185 patients with symptoms suggestive of tuberculosis underwent a standardised clinical assessment and provided 299 sputum samples that were tested for TB by all diagnostic techniques. Costs indicate consumables only, per sample. Patients were considered to have tuberculosis if any culture test was positive. Culture-negative patients were followed up for five years to determine whether PCR-positive but culture-negative patients represented false-positive PCR or false-negative cultures.

Results: The performance of each diagnostic approach was analysed by patient, including testing of multiple samples (graph):

- Symptomatic predictors of a positive TB culture were night sweats (p=0.03); fever (p=0.03); and weight loss (p=0.045) with sensitivity 74%, 67% and 83%, and specificity 46%, 53% and 35%, respectively. Combining symptoms with Boolean functions identified [night sweats & weight loss] as the best predictor of TB, sensitivity/specificity 63%/61% respectively for microscopy-negative patients.
- Ziehl-Neelsen light microscopy had 100% specificity and 48% sensitivity (cost ~$0.1 per sample).
- Auramine fluorescence microscopy had 99% specificity and 70% sensitivity (~$0.5).
- The IS6610 PCR technique had 91% sensitivity, 87% specificity and cost >$5. Seventeen patients were PCR positive but negative by all other tests. Epidemiological assessment determined that, relative to patients for whom all tests were negative, these patients with isolated PCR-positive results were no more likely to have had previous TB disease or contact with a TB patient, be tuberculin skin test positive, die, nor were they or their household contacts more likely to develop TB over the subsequent five years. Consequently, these 17 isolated PCR-positive results were considered to be false-positive tests.
- Lowenstein-Jensen culture (~$0.1) had 87% sensitivity, 26% contamination rate and median time to positive culture of 22 days.
- MODS, (~$1-2) had 96% sensitivity and compared with Lowenstein-Jensen culture, had less contamination (4%; P<0.0001), and more rapid detection (median 7.5 days; P<0.0001). Delay between sample collection and culture increased contamination rates in Lowenstein-Jensen (p=0.02) but not MODS cultures (p=0.97).
- Availability of second samples increased the sensitivity of microscopy (Ziehl-Neelsen by 33% and auramine by 30%; both P>0.05) but had little effect on the sensitivity of PCR (0%) or of culture (LJ 9.5%, MODS 6.7%).
- MODS provided direct antibiotic susceptibility results at the same time as culture detection, over five weeks sooner than indirect susceptibility MABA testing (~$5). Susceptibility concordance was 100% with the MABA assay, but all strains were drug susceptible.



Conclusion: Symptom scores did not reliably differentiate between patients whose symptoms were caused by tuberculosis vs. other diseases. PCR had an unacceptable false-positive rate. A single MODS sputum culture doubled tuberculosis diagnostic sensitivity compared with repeated Ziehl-Neelsen microscopy, and was significantly more rapid and sensitive than Lowenstein-Jensen culture.

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Diagnostic Utility of LED Fluorescence Microscopy to Detect Acid-Fast Bacilli in Sputum

Brittle W[1], Painczyk K[1], Hesseling A[1], Warren RM[2], Beyers N[1], Wasserman E[3], van Soolingen D[4], Marais BJ[1,5]

Aims: To evaluate the diagnostic performance of light-emitting diode (LED) fluorescence microscopy, as alternative to the mercury vapour lamp and to compare its performance to conventional light microscopy using Ziehl-Neelsen (ZN) staining

Design: A prospective, blinded, laboratory-based study

Methods: Routinely collected sputum smears were stained with Auramine-O and analysed microscopically using two different light sources (mercury vapour lamp vs LED). Two microscopists, blinded to previous readings, evaluated all smears independently. Subsequently, smears were ZN stained and reread using standard light microscopy. Time spent reading each smear was documented. The performance of the different light sources and staining methods was calculated relative to bacterial culture results obtained for the same specimen.

Results: 221 sputum specimens were evaluated, of which 33 (14.9%) were smear-positive. The combined sensitivity and specificity for microscopy was: LED 84.7% and 98.9%, Mercury 73.6% and 99.8%, ZN 61.1% and 98.9%, respectively. The percentage agreement for positive smears was: LED 87.9 % (Kappa 0.87); Mercury 88.0% (Kappa 0.79); ZN 82.6% (Kappa 0.77). The average time spent reading a negative smear was 1.4 and 3.6 minutes for fluorescence and light microscopy respectively (no difference between LED and Mercury).

Conclusion: LED fluorescence microscopy provides an inexpensive and time-saving alternative to conventional methods.

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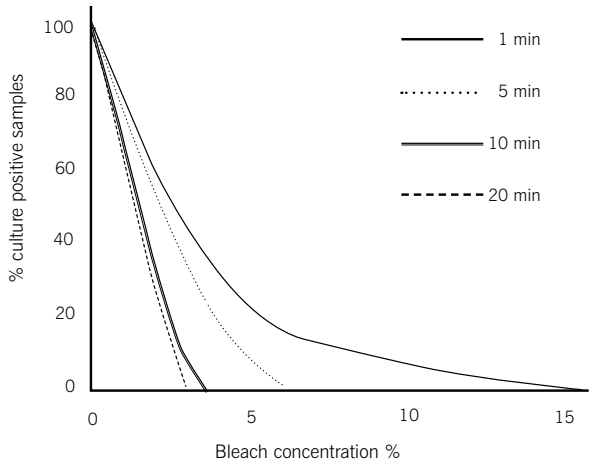
Sputum Bleach Sedimentation improves the Safety and Speed of Microscopy for Tuberculosis Diagnosis

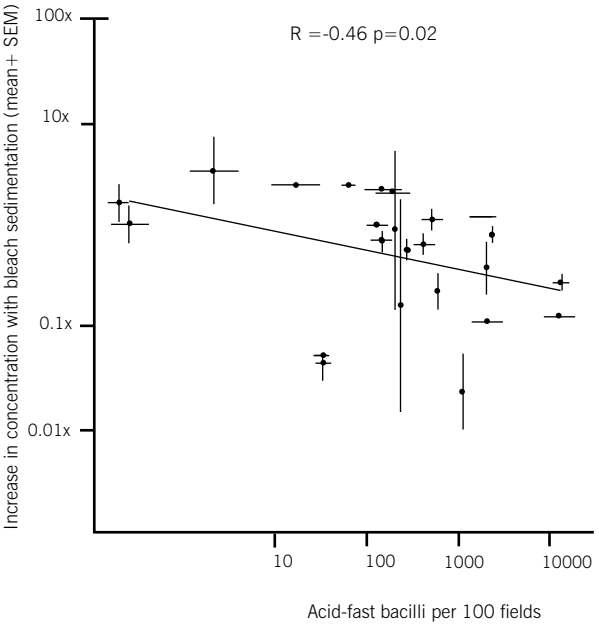
Rusheng Chew,[1] Carmen Calderón,[2] Robert H. Gilman,[2,3] Jonathan Sherman,[4] Luz Caviedes,[2] Patricia Fuentes,[2] Jorge Coronel,[2] Teresa Valencia,[2] Beatriz Hererra,[2] Mirko Zimic,[5] Lucy Huaroto,[6] Ivan Sabogal[7], A. Rod Escombe,[2,8] Carlton A. Evans[2,8]

Background: Tuberculosis diagnosis by direct sputum smear microscopy is rapid and inexpensive but insensitive, sensitivity being typically only 30-50% per sputum sample in operational settings. Bleach-sedimentation has been proposed to sterilise and concentrate mycobacteria into the small sputum volume visualised by microscopy, improving sensitivity, reading speed and safety. However, the safety and efficacy of sputum bleach-sedimentation is poorly defined, with past studies being only qualitative in nature.

Objective: To quantitatively evaluate the sputum bleach-sedimentation technique in terms of its mycobactericidal activity, effect on slide-reading efficiency and ability to concentrate mycobacteria.

Methods: 55 clinical sputum specimens from newly-diagnosed tuberculosis patients were used in this study. To assess sterilising efficacy, 31 sputum samples were exposed to 16 varying bleach concentrations and exposure times. The remaining 24 sputum samples were processed with the following gravity bleach-sedimentation technique. To 1 ml of sputum, an equal volume of fresh 5% bleach was added, and the mixture shaken by hand for 10 minutes. Distilled water was then added to 10 ml and the mixture left to sediment for 16 hours. The supernatant was pipetted off and the pellet, or the basal 250 μl if no pellet had formed, was resuspended in the remaining fluid. As a standardisation measure, 40 μl of sputum was used to make each smear, which covered an area of 1×2 cm. Using oil-immersion microscopy, the number of mycobacteria/100-300 fields was counted in a blinded manner for 144 smears in triplicate pre- and post-bleach sedimentation. Results: All samples were sterilised by five minutes' exposure to 6% bleach or 20 minutes' exposure to 3% bleach (Figure, left). Bleach-sedimented slides were read more rapidly than control slides (9.6 vs. 11.2 minutes, p=0.03). There was good inter-observer agreement but bleach made it difficult to identify the stained area, thus three bleached slides vs. no control slides were misread as negative by one technician (p=0.2). Bleach-sedimentation caused a decrease in mycobacterial counts (p=0.05). However, this decrease was significantly less than the 10-fold dilution required by the methodology (p=0.001), demonstrating that sedimentation caused partial concentration of mycobacteria. Bleach-sedimentation caused less dilution of sputum that contained lower concentrations of mycobacteria (p=0.02).





Conclusions: This research demonstrates a controlled methodology for optimisation of bleach-sedimentation. Bleach-sedimentation decreased the concentration of mycobacteria for detection by microscopy but these data support the use of this technique to improve laboratory safety and efficiency.

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Evaluation of the Capilia TB Assay and the GenoType Mycobacterium Assay to Identify MTB Complex Directly on Liquid Culture (MGIT)

Petra de Haas[1,2], Monde Muyoyeta[2,1], Winnie Mwanza[2], Pike Mwamba[2], B. Ruth Tembwe[3], Helen M Ayles[1,2]

Background: With the use of more sensitive liquid culture techniques there is increasing need for a cheap, rapid and robust identification test for bacteria of the M.tuberculosis complex. The Capilia TB assay (TAUNS) is based on immuno-chromatographic detection of the secretion protein Mpb64.

Mpb64 is specific for M.*tuberculosis* complex isolates except for some sub-species of *M.bovis* BCG. The test can be performed directly on a positive MGIT culture and can be read within 20 minutes.

Objective: To test the sensitivity and specificity of Capilia TB test compared to the Genotype Mycobacterium CM assay (HAIN LifeSciences) on prevalence survey samples in Zambia.

Methods: In 2005 a prevelance survey of over 8000 non-symptomatic individuals was conducted in two communities in Zambia. This survey found a prevalence of tuberculose of about 1% and 5.6 % mycobacteria other then tuberculosis complex species (MOTT) were found. A selection of 161 isolates from this study was used to evaluate the value of the Capilia TB test compared to the Genotype mycobacteria CM assay (CM) for the identification of M. *tuberculosis.* The CM assay is a PCR based test using the reversed line blot method to identify at once *M. tuberculosis complex* and the most common MOTT species. Both assays were performed according to manufacturers' protocols.

Results: In total we found 73 M.tuberculosis complex isolates, 43 *M.intracellulare, 6 M.scrofulaceum, 4 M.fortuitum, 2 M.gordonae, 3 M.interjectum, 3 M.peregrinum* and 27 MOTT isolates that could not be identified using the CM assay.

There is good agreement between the CM and Capilia TB test. The sensitivity and specificity of the Capilia TB assay are both 99.4.%. One isolate that was Capilia TB negative but *M.tuberculosis* complex according to CM was found to be *M.tuberculosis* on spoligotyping and one isolate was identified as *M.tuberculosis* complex by Capilia TB test but was found to be an unidentified MOTT according to the CM assay. This culture is ZN positive with no clear cording. Further identification is needed. Whitin the MOTT isolates 69% of the strains could be speciated using the GenoType mycobacteria CM assay.

Conclusion: This study demonstrates that the Capilia TB test is an easy, robust and rapid test to identify *M. tuberculosis* complex from culture. It is less time-consuming then Genotype mycobacterium CM assay and does not need special laboratory equipment. The advantage of the Genotype Mycobacteria CM assay compared to the Capilia TB test is that at once you can specify species within the MOTT isolates. Furthermore Genotype mycobacteria assay has a negative contol built in whereas Capilia TB test does not.

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Diagnostic Accuracy of the SH-HS Method for AFB Smears and Culture

Christian A. Ganoza[1], Jessica N. Ricaldi[1], José Chauca[1], Gabriel Rojas[1], César Munayco[2], Juan Agapito[3], Juan Carlos Palomino[4] and Humberto Guerra[1]

Setting: Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, and Hospital Nacional Cayetano Heredia, Lima, Perú.

Objective: To evaluate a new decontamination and concentration method for sputum microscopy and culture.

Design: Sputum samples from patients with suspected pulmonary tuberculosis (TB) (N=106) were tested using the proposed Hypertonic Saline-Sodium Hydroxide (HS-SH), the recommended N-Acetyl-L-Cysteine - Sodium Citrate - Sodium Hydroxide (NALC-NaOH), and the direct smear (Ziehl-Neelsen) techniques for the presence of mycobacteria using culture (Lowenstein-Jensen, L-J) and light microscopy.

Results: From the 106 collected samples, 12 were excluded because of culture contamination by bacteria other than *Mycobacterium* (13/212 cultures, 6.1%). Of 94 valid specimens, 21 (22.3%) were positive in culture. The sensitivity for acid-fast bacilli (AFB) smears was significantly increased using the decontamination and concentration methods (see table). Both concentration techniques were highly comparable for culture (Kappa [K] = 0.794) and smear (K = 0.631) for AFB.

Table: Sensitivity, Specificity and Efficiency of AFB Microscopy with each method, compared to Total Positive Cultures*

	HS-SH	NALC-NaOH	Direct smear (NTP)
Sensitivity (95% CI)	71.4% (52.1–90.8)	66.7% (46.5–86.8)	28.6% (9.2–47.9)
Specificity (95% CI)	87.7% (80.1–95.2)	87.7% (80.1–95.2)	100% (100–100)
Efficiency (95% CI)	84% (76.6–91.4)	83% (75.4–90.6)	84% (76.6–91.4)
PPV (95% CI)	62.5% (43.1–81.9)	60.9% (40.9–80.8)	100% (54–100)
NPV (95% CI)	91.4% (80.2–100)	90.1% (78–100)	83% (73.5–90)
Culture-positive detected/non-detected**	15/6	14/7	6/15

* Upper 95% CIs exceeding 100% were rounded to 100%; PPV=Positive Predictive Value, NPV=Negative Predictive Value.

**X^2= 9.39, DF=2, p<0.01; Difference of the Proportions for sensitivity (95% CI): HS-SH vs NTP's direct method= 0.11-0.75 (Z=2.47, p=0.014), NALC-NaOH vs NTP's direct method= 0.05-0.71 (Z=2.16, p=0.031), NALC-NaOH vs HS-SH= -0.37–0.28 (Z=0, p=1)

Conclusion: The proposed HS-SH method improves the sensitivity for AFB microscopy and *M. tuberculosis* culture; its performance is comparable to the NALC-NaOH method, but it is methodologically simpler and less expensive, making it a good candidate for evaluation by national TB programmes in developing countries.

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The Colorimetric Indicator STC Accelerates Tuberculosis Culture Diagnosis

Jessica Alvarado[1,4], Willi Quino[1], Robert H. Gilman[1,2,4], Eric Ramos[1], Beatriz Herrera[1], Scarlet Shell[3], Teresa Valencia[1], Rosario Montoya[1,4], Jessica Alva[1,4], Jessica Franco[1,4], Maria Haro[1,4], Rosario Sosa[1,4], Enit Valera[1,4], Betty Valiente[1,4], Gurjinder Sandhu[5], Maribel Rivero[1,4], Silvia Carrera[1,4], Antonino Curatola[1,4], A. Roderick Escombe[5], Paty Sheen[1], Carlton A. Evans[1,2,4,5]

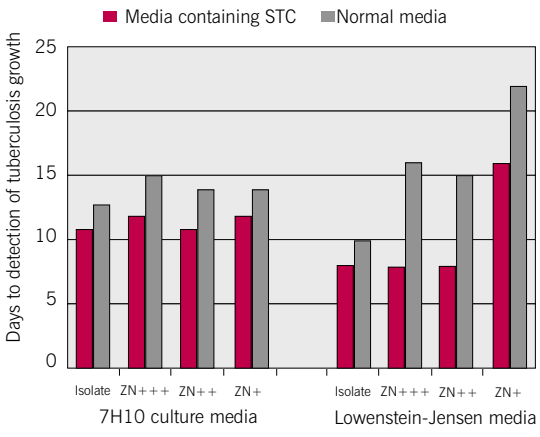
Background: Tuberculosis (TB) diagnosis by culture is especially important for children, people living with HIV-infection and for multi-drug resistant TB testing. The most widely-used media are egg-based (e.g. Lowestein Jensen, LJ), that are usually prepared locally, and more expensive artificial Middlebrook media (e.g. 7H10). TB colonies are a similar colour to the media, making them difficult to see, especially for large-scale testing. TB growth takes several weeks to be visible with the naked eyes and this delay impairs patient care. STC (2,3-diphenyl-5-thienyl-(2)- tetrazolium chloride) is a colorimetric indicator that is stable in incubators and changes colour when micro-organisms grow.

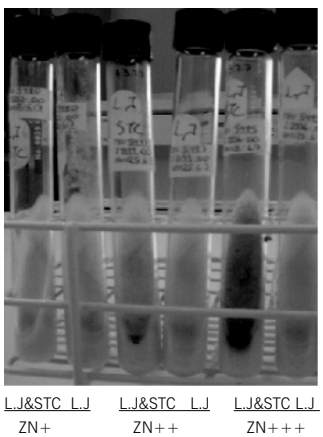
Objective: To evaluate incorporation of the colorimetric indicator STC into media used for TB diagnosis.

Methods: Standard LJ, 7H10, Ogawa, 7H11 solid media and 7H9 broth media were prepared with and without 50 μg/ml STC in the culture media. Clinical sputa with Ziehl Neelsen (ZN) smear microscopy grades +, ++ and +++ and also a laboratory isolate of the strain H37RV were decontaminated using sodium hydroxide and inoculated onto the media in parallel in a blinded manner. Cultures were then all permanently double-sealed and incubated in air at 37ºC. Cultures were examined by naked eye 3x/week. Raw data are presented, without adjustment for intermittent inspection. Data means (and standard deviations, SD) are shown that were compared by paired students' T-test. Speciation was determined by colony morphology.

Results: Colonies caused a red colouration of STC that was visible to the naked eye in all culture media. There was no augmentation or inhibition of TB growth by STC. Quantitative evaluation is complete for LJ and 7H10 media (graph). In 7H10, TB growth was visible an average of 3 (SD 1) days sooner with STC than without ($P<0.005$). In LJ medium, TB growth was visible 6 (SD 3) days earlier with STC than without ($P<0.05$, photograph). Preliminary results with 7H9 and 7H11 are similar and Ogawa evaluation is in progress. The bright red colouration of the STC-containing media surrounding each colony facilitated identification of positive cultures. Cultures contaminated by bacterial or fungal overgrowth also caused the STC containing media to change colour, but colony morphology distinguished this from TB growth. Cultures were interpreted without being opened, facilitating bio-safety.

Conclusions: The colorimetric indicator STC increased the speed and ease of the most widely used tuberculosis culture techniques. Large-scale evaluation is in progress. STC may be incorporated into TB culture media to facilitate laboratory processes and accelerate patient diagnosis and is currently being tested for concurrent colourimetric multi-drug resistant TB testing.





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Comparison Between Lowenstein Jensen and MIGIT 960: Recovery and Time Rates

Gutierrez M Vicente A, Montoto M, Hoffman M

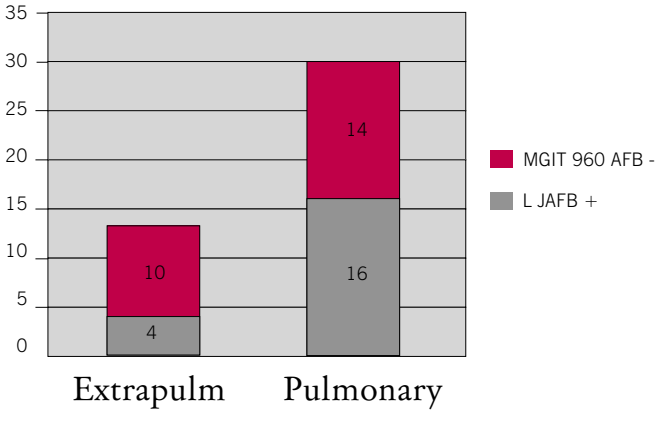
Making a quick diagnosis of pulmonary tuberculosis (TB), continues to be a major challenge in the control of this ancient disease. Resurgence of TB worldwide has been accompanied by an increase in the incidence of multidrug resistance tuberculosis (MDR Tb), and extended resistance tuberculosis (XDR Tb). Growth of MTbc on traditional Lowenstein Jensen (LJ) media requires more than four weeks, which represents a delay in accessing the appropriate treatment (with all the epidemiological problems that this implies).

In Argentina, more than 13 000 new TB cases are reported every year: we're still far from getting TB under control. We work at the Mycobacteriology Laboratory of Hospital Enrique Tornú, in Buenos Aires, Argentina. This is one of three Mycobacteria Reference Laboratories in Buenos Aires. Between 1/1/2006 and 1/1/2007 we processed a total of 4325 tests, of which 856 yielded a positive culture (19.7%). Culture aport to TB diagnostic: nearly half of the total bacteriological TB diagnostics were made only through culture, since those specimens had a negative bacilloscopy. Of the total materials that yielded culture positive for mycobacteria, with a negative AFB stain (n=386), 205 were recovered from MGIT960 system (53%) and 181 from L J (47%). There were 44 materials that could be recovered only with MGIT 960 system: 14 from extrapulmonary sites and 30 respiratory specimens. This represents 5.1% of the total number of positive cultures.

In fig 1 we can see the details of these 44 materials:

TB bacteriological confirmation couldn't be made in 24 specimens that had a negative bacilloscopy and didn't grow in LJ media but had a positive culture only with the MGIT 960 system. Average time to detection: LJ: 24 days and MGIT 960: 12 days. Rapid diagnosis of mycobacterial infections is critical therefore attempts to shorten the time needed for detection deserve full attention. MGIT 960 system has been demonstrated to have two important advantages over LJ media: it has better performance, not only regarding recovery rates, but also in detection time. There is no doubt about the importance of making a quick and accurate TB diagnosis in order to avoid thousands of new cases each year. This, combined with prevention, is the only way to win the battle against TB; that is why it is critical to add a liquid media such as MGIT 960 to traditional LJ.

Figure 1.



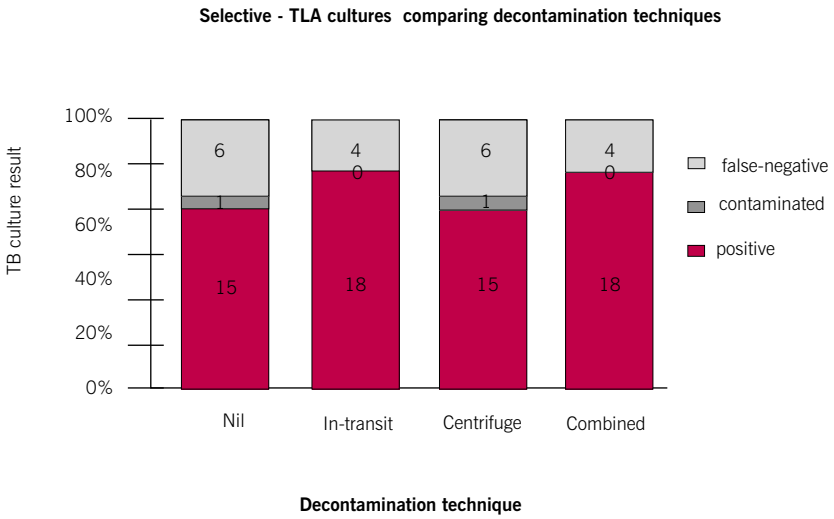
Mycobacteriology Laboratory, Hospital Enrique Tornú, Buenos Aires, Argentina

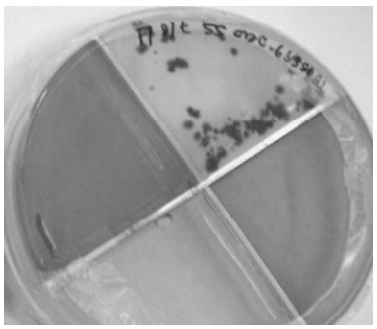
Optimisation of TB Field Testing: In-Transit Sputum Decontamination & Culture on Colorimetric Selective Media for TB Diagnosis & Drug-Susceptibility Testing

Beatriz Herrera[1], Eric Ramos[1], Robert H. Gilman[1,2,3], Louis Grandjean[2], Laura Martin[2], Jessica Alvarado[1,2], Willi Quino[1], Teresa Valencia[1], Gurjinder Sandhu[1,2,3], Rosario Montoya[1,2], Jessica Alva[1,2], Jessica Franco[1,2], Maria Haro[1,2], Rosario Sosa[1,2], Enit Valera[1,2], Betty Valiente[1,2], Maribel Rivero[1,2], Silvia Carrera[1,2], A. Rod Escombe[4], Antonino Curatola[1,2], Carlton A. Evans[1,2,3,4]

Background: TB particularly afflicts disadvantaged populations. Consequently, reference laboratories and the technologically demanding tests for MDRTB that they provide are least available to those in greatest need. In endemic settings, salivary micro-organisms usually overgrow sputum samples during transit to the laboratory where they are then killed by decontamination with strong alkali. This decontamination also kills most of the TB, reducing sensitivity, and largely restricting the use of TB culture to bio-secure laboratories. The thin-layer agar (TLA) technique has the potential for field use for TB diagnosis and MDRTB testing but the requirement for sputum decontamination hampers implementation in field laboratories. We aimed to optimise sputum processing and culture for field use.

Methods: Quantitative studies of TB colony numbers and time to growth were used to optimise for TLA, an 'in-transit' liquefaction and decontamination transport medium for field use. This single-step transport medium (Trisodium phosphate, ammonium sulphate, magnesium sulphate, ferric ammonium citrate, penicillin) is stored at room temperature. Sputum is expectorated directly into a sputum pot containing the solution that kills contaminating salivary micro-organisms while the sample is in transit to the laboratory, without killing the TB within the sample. The TLA procedure was modified with antimicrobial-enriched culture media that discourages contamination (Selectatabs). The media also incorporated a colorimetric indicator of microbial growth (2,3 diphenyl-5-(2-Thienyl) Tetrazolium chloride) STC) that facilitates culture interpretation. Newly-diagnosed patients with pulmonary tuberculosis expectorated similar volumes of sputum collected at the same time directly into two sputum pots, a normal dry pot and another containing transport medium that was stored overnight at room temperature at an inclined angle, to sediment TB. The 'sediment' or lowest part of the sputum from both pots (with and without transport medium) was then inoculated directly onto culture medium. The remainder of both samples were then processed with standard laboratory sodium hydroxide centrifuge decontamination and then cultured in the same way. All cultures were done on Petri-dishes containing Middlebrook 7H11 culture medium supplemented with 10% OADC, Selectatabs, 50μg/ml STC. One un-supplemented quadrant was used for detection and other quadrants were supplemented with isoniazid and rifampicin. The fourth quadrant was used for exploratory ciprofloxacin research. Immediately after inoculation, all cultures were double-sealed with tape within a 'ziplock' transparent plastic bag and were incubated in room air, without C02, at 37°C. Positive cultures were identified by naked-eye colour change and speciation was confirmed by morphology using x40 magnification examination of the double-sealed cultures with a normal laboratory microscope. Usually the double-sealed cultures would be read by microscope and then





Sputum collected directly into in-transit decontamination transport medium was applied to selective-TLA media. Growth was visible by naked-eye colouration of the STC indicator (red dots in the upper clear quadrant.) Other quadrants contain isoniazid (green), rifampicin (yellow) & ciproflozacin (blue). TB did not grow concurrently in these drugs, demonstrating that TB was susceptible to them.

destroyed without opening, but for this experiment colonies were instead extracted to confirm the drug-susceptibility results with the TEMA assay.

Results: To date, results are complete for 22 patients who were all smear microscopy positive (see Figure). 15/22 (68%) were sputum culture-positive by standard testing with laboratory centrifuge-decontamination whereas 18/22 (82%) were culture-positive with the 'in-transit' decontamination. Median (IQR) days to culture results were 17 (14-26) with in-transit decontamination, 18 (15-31) with no decontamination, 26 (18-30) for laboratory centrifuge-decontamination and 19 (14-31) days for both decontamination techniques combined. Culture speed did not differ significantly between decontamination techniques. The direct drug-susceptibility testing results were read the same day as TB was detected (see photograph) and these isoniazid and rifampicin results were completely concordant with the indirect TEMA testing that became available 1-2 months later (n=10 so far).

Conclusions: This ongoing evaluation suggests that in-transit decontamination combined with selective TLA media, a colorimetric indicator and direct MDRTB testing may be applicable in field settings without biosafety cabinets, because setting up these cultures involves equivalent bio-hazard to sputum smear microscopy, after which the cultures are permanently double-sealed until disposal. The simplicity and safety of this technique has the potential to make MDRTB diagnostic testing more widely available in resource-poor settings.

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Improvement of Tuberculosis Case Detection and Reduction of Discrepancies Between Men and Women by Simple Sputum-Submission Instructions: A Pragmatic Randomised Controlled Trial

Mishal Khan[1], Osman Dar[2], Charalambos Sismanidis[1], S. Karam Shah[3], Peter Godfrey-Faussett[1]

Background: Detecting patients whose sputum is smear-positive on microscopic examination is one of the cornerstones of the public health strategy to control tuberculosis. It is estimated that only 53% of the estimated smear-positive cases were notified under DOTS in 2004[1, 2], indicating that a significant proportion of smear-positive cases are going undetected within DOTS areas. Recent studies have suggested that women, in particular, are at risk of under-detection; in several settings, female tuberculosis suspects test smear-positive less frequently than males[3,4,5]. Submission of poor quality sputum specimens by women may be one reason for the gender difference.

Methods: We conducted a pragmatic randomised controlled trial to evaluate the impact of sputum submission instructions on patients at the Federal Tuberculosis Centre, Pakistan. 1 494 females and 1 561 males suspected of having tuberculosis were randomised either to receive sputum submission guidance prior to specimen submission or to submit specimens without specific guidance, according to prevailing practice. The primary outcome measure was the proportion of females testing smear-positive. Secondary outcome measures were the proportion males testing smear-positive, the proportion of saliva specimens submitted by males and females, and the proportion of males and females returning the next day with an early morning specimen to complete the diagnostic procedure.

Results: Instructed females were far more likely to test smear-positive than those in the control group. Using a case definition of two specimens positive, instructions resulted in a 63% increase in smear-positive case detection (8% in the control arm vs 13% in the intervention arm (p= 0.002). Under the newer WHO TB case definition of one specimen positive, instructions resulted in a 46% rise in smear-positive case detection (10% in the control arm vs 14% in the intervention arm (p= 0.007). Instruction was associated with a decrease in spot-saliva submission (p=0·003), and an increase in the number of women returning with an early-morning specimen (p=0·02).

Men in the instructed group also showed increased smear-positivity and improvement in specimen quality. However, the magnitude of the improvement was smaller in men than in women and was not significant.

The cost per extra case detected was 129 PKR (which is about US$2).

Interpretation: In the Federal Tuberculosis Centre in Rawalpindi, lower smear-positivity previously recorded in women was mainly a function of poor quality specimen submission. Substantial improvements in case detection of women were achieved by brief instructions about the importance and technique for producing a good quality sputum specimen.

The biggest factor leading to increased smear-positivity in instructed females was probably a reduction in poor quality (salivary) specimen submission. The effect of instructions on specimen quality was most likely greater in women in this setting because they were less knowledgeable about the difference between sputum and saliva and the need to submit a sputum sample for diagnosis. It is also likely that men, in general, are physically more able and comfortable about expectorating sputum. Instructions also appeared to improve the yield from sputum (good quality) specimens submitted and significantly increased the proportion of women returning with a second specimen to complete the diagnosis process. These factors also played a role in the increased smear-positive case detection resulting from the provision of instructions.

We emphasise that the intervention was designed to be cheap and easily replicable in low income countries; implementation only required a private space for instructions and a trained female health worker to provide approximately two minutes of guidance to each patient. Training the health worker to deliver instructions only took half a day and the cost per extra case detected was extremely small. Therefore, sputum-submission guidance might be a highly cost-effective intervention to improve smear-positive case detection and reduce the disparity between the sexes in tuberculosis control in low-income countries.

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Footnotes:

1. WHO. Global tuberculosis control surveillance, planning, financing: WHO report 2005
2. Dye, C. et al. 'What is the limit to case detection under the DOTS strategy for tuberculosis control?' Tuberculosis (Edinb) 2003; 83(1-3):35-43
3. Boeree, M.J. et al. Gender differences in relation to sputum submission and smear positive pulmonary tuberculosis in Malawi. Int J Tuberc Lung Dis 2000; 4(9):882-4
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7H9 Broth is an Ideal Tuberculosis Culture Medium for Resource-limited Countries

M.K. Kaswa*[1,2], S.C. Larsen[1], A. Clobridge[1], A. Somoskovi[1], M. Salfinger[1]

Growth detection of M. tuberculosis (MTBC) is still indispensable since culture is more sensitive than microscopy and conventional drug susceptibility tests require viable organisms. The introduction and routine application of commercially available broth-based culture systems might not be applicable or affordable for laboratories in resource-limited countries. Therefore, a prospective study was organised to evaluate the performance of the inexpensive, home made 7H9 broth for the recovery rate and time to detection of MTBC and to compare the results with those of the MGIT 960, BACTEC 460TB and Lowenstein-Jensen (LJ) media. The 7H9 tubes were weekly centrifuged, an aliquot stained, and considered positive when cord formation was detected. A total of 106 MTBC isolates were recovered from 136 clinical specimens from known TB patients. The rates of recovery of MTBC were 99% with 7H9, 85.6% with both the MGIT 960 and the BACTEC 460TB, and 70.5% with the LJ. The mean time to detection of MTBC in smear-positive specimens was 8.8 (4-18) days for 7H9, 9.4 (2-24) for MGIT 960, 8.3 (2-19) for BACTEC 460TB, and 21.3 (14-35) for LJ, and in smear-negative specimens, it was 14.0 (4-42) days for 7H9, 14.2 (6-18) for MGIT 960, 16.3 (2-53) for BACTEC 460TB, and 26.0 (14-35) for LJ.

In conclusion, the 7H9 broth can be considered a viable alternative to shorten the TAT for growth detection while increasing the yield of MTBC compared to LJ, BACTEC 460, and MGIT 960 in resource-limited countries.

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Rapid Tests for Detecting MDR-TB in Kampala, Uganda

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As part of a larger project to investigate strategies for controlling MDR-TB in Uganda we are evaluating rapid detection of drug resistance directly from sputum. The first phase of the project was a pilot study to assess the feasibility of implementing technology for rapid detection of in MDR-TB in Kampala.

Objective: To assess the performance, turn-around time and costs of four tests for resistance to rifampicin when applied to smear positive sputum. Test accuracy, cost per sample and turn-around times were compared.

Study design: Smear positive specimens from re-treatment TB patients were tested for resistance to rifampicin by four technologies.

Liquid culture	BACTEC 460 (Becton Dickinson, USA)	(n=39)
Bacteriophage replication assay	In-house phage assay	(n=77)
Genotypic detection of mutations	INNO-LiPA Rif.TB, (Innogenetics, Belgium)	(n=43)
Drug impregnated strips	Etest, AB Biodisk, Solna, Sweden	(n=44)

Results were compared to those obtained testing the cultured isolates by BACTEC 460.

§ Due to the small number of tests performed in this pilot study performance data is not statistically validated.

* Spoilt tests due to technical failure or contamination.
# LiPA tests were batched which reduced labour costs but increased time to result.

Conclusions: Direct detection of rifampicin resistant tuberculosis in smear positive sputum is possible in this setting and may be more cost effective than the current method of indirect testing.

	Failed tests* (%)	Concurrence with indirect test§ (%)	Days to result (mean)	Cost reagents (USD)	Cost labour (USD)	Recurrent costs (USD)
Indirect BACTEC	9		8-60 (24)	30.01	11.65	41.66
Direct BACTEC	13	100	4-20 (8)	22.21	6.41	28.62
Direct Phage	27	96	2-4	11.20	8.20	19.40
Direct LiPA	14	93	2-4#	36.49	5.43#	41.92
Direct ETest	11	100	14-27 (20)	12.56	6.04	18.60

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A Rapid Microcolony Susceptibility Test

Denis A. Mitchison, David Coleman

There is a need for a rapid direct susceptibility test (DST) that can be applied to all anti-TB drugs and that is also cheap to run. Such a test is being developed, based on microcolonies growing on selective 7H11 oleic acid albumin agar plates containing polymyxin B sulphate 200 U/ml, ticarcillin 100 μg/ml, trimethroprin lactate 20 μg/ml, and amphotericin B 10 μg/ml (obtainable from Mast as Mycobacteria selectatabs Kirchner M24). Six to eight separate strains of *M. tuberculosis* can be inoculated on to sectors of a single plate. Single plates with the following drug concentrations can then be inoculated: isoniazid 0.2 μg/ml; rifampicin 0.5 μg/ml; ethambutol 2.0 μg/ml; streptomycin 4.0 μg/ml; ofloxacin 0.25 μg/ml; moxifloxacin 0.5 μg/ml; nicotinamide 500 μg/ml (for pyrazinamide sensitivity) and sodium p-nitrobenzoate 500 μg/ml (to confirm identification). Used as an indirect test on cultures, the results can be read after incubation for seven days. The plates are sealed for safety with 'shrink-seals'. The growth of microcolonies is best seen using an inverted microscope with a long distance, low power x20 objective, preferable with phase contrast available. Direct tests are under development. Sputum is homogenised with dithiothreotol (DTT) and is then inoculated directly on to segments of the plate. The biggest problem with the direct test is a rather high contamination rate, in our experience affecting 10% of sputum samples. Work is proceeding on improving the selective culture medium.

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Performance of Different Culture Systems for Isolation of TB and Implications for TB Control In High TB and HIV Endemic Areas

Monde Muyoyeta[1], A. Schaap1, Ruth Tembwe[3], Winnie Mwanza[1], Petra De Haas[1], D. Milimo[1], P. Godfrey-Faussett[2], H. Ayles[1,2]

Introduction: There is increasing pressure for poorly-resourced countries to extend the use of culture to diagnose TB. The MGIT system is known to have a higher yield and quicker time to recovery of TB but is largely not available in resource-limited countries. The ZAMSTAR study is evaluating the routine use of MGIT culture on clinical samples that are received at the National Reference Laboratory in Zambia.

Methods:

- Samples digested and decontaminated using NaOH and NALC system.
- Samples split four ways and inoculated on commercial LJ (CLJ), home-made LJ (HLJ), MGIT 960 (AMGIT) and manual MGIT (MMGIT).
- ZN stain positive isolates subjected to identification tests to confirm presence of *Mycobacterium tuberculosis complex*.

Results: 375 Mycobacterium tuberculosis complex (MTB) were isolated in total. AMGIT isolated 313 (83%) MMGIT 320 (85%), CLJ 176 (47) HLJ 170(45%). 147 non-tuberculous mycobacteria (NTM) were isolated, MMGIT recovered 77 (52%), AMGIT 70(48), CLJ 13 (9%) and HLJ 10 (7). Of 134 smear negative samples, CLJ isolated 58 (43%), HLJ 50(37 %), AMGIT 88 (66%) and MMGIT 91 (67%). The median time to positivity for smear negative samples was 16 days on AMGIT, 18 days on MMGIT and 34 days on both LJ media.

Discussion: MGIT systems recovered more Mtb from smear negative samples than both LJ systems. The MGIT system was also quicker to give results. Using MGIT system may improve the diagnosis of TB in high HIV endemic areas that have seen an increase in smear negative TB.

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Improving on Sputum Collection and Diagnosing Tuberculosis in the Field

E.F Onyoh[1,2] and E. Kongnyuy[1]

Aim: This study was to determine an improved method of collecting adequate sputum samples from patients suspected of having Pulmonary Tuberculosis (PTB) and Disseminated TB (DTB) in the field especially in HIV/TB co-infected patients and children who were unable to produce adequate samples for smear microscopy using hypertonic saline nebulisation in Cameroon.

Background: Diagnosis of TB is traditionally based on identification of M. tuberculosis by microscopy or culture of relevant patient material (e.g. sputum, lymph node aspirate, etc). However, microscopy is labour intensive, not very sensitive and prone to error under field conditions, while culturing of bacteria can take several weeks before the result is ready. Furthermore, at the field level a substantial number of TB patients are negative by microscopy and Mantoux test might not even be suggestive, especially in immuno-compromised patients. Adequate sputum collection for analysis might not be possible. So the diagnosis of these patients is complicated and often leads to delayed treatment due to lack of a better diagnostic procedure. Improved and specific tests for use in diagnosis of TB at the field are therefore urgently needed. While waiting and hoping for a better field diagnostic test we decided to improve on our collection of sputum in suspected TB infected individuals who were unable to produce adequate sputum for microscopy.

Methods: This is an uncontrolled observational study (case series) done at our peripheral hospital involving 64 individuals suspected of being infected with Mycobacterium tuberculosis between January 2006 to June 2006. Suspicion was raised following clinical assessment, Mantoux test (equivocal), traditional daily single sputum for three days ('inadequate' volume) with no AFB seen on microscopy. We felt maybe we were not obtaining enough sputum containing Mycobacterium tuberculosis. In order to improve on this method we decided to nebulise our subjects with 35 ml of 3.0% hypertonic saline and after about an hour we got about 5-20ml of sputum. The samples were immediately taken to the laboratory for microscopical analysis. This procedure was repeated twice daily (five hours apart) on days one and two, and once on day three. Gastric washing (GW) was also performed on some of these patients once daily in the morning (before breakfast) on days one, two and three. The small amount of washout collected using nasogastric tubes was later sent to the laboratory for microscopy. More than half of the 64 patients refused gastric washings.

Results: Sputum Induction produced more volume of sputum compared to gastric washings. About 35% of the patients were found to be positive for Acid Fast Bacilli after microscopy from hypertonic saline nebulisation. The remaining 65% were either placed on antibiotics and given appointments for follow up or referred to the city for further evaluation and investigations, while a few were treated as smear negative PTB. The 35% above could have been missed if this extended method had not been performed.

Discussion: In suspected pulmonary TB patients who were unable to expectorate sputum spontaneously, the diagnostic yield of five sputum samples induced by nebulised hypertonic saline was greater than that of GW specimens obtained on three consecutive days, and more patients were diagnosed (35% additional) and started DOTS immediately. Hence, there was an increased in number of cases detected following sputum induction with 3.0% hypertonic saline nebulisation. However, there is a dire need for a better tuberculosis diagnostic method in the field which is where the majority of our TB patients are living.

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Comparative Evaluation of BACTEC MGIT 960 System in The Gambia

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Background: Tuberculosis (TB) continuous to be a leading cause of mortality and morbidity worldwide. It is a serious re-emerging infectious disease that affects the lungs and respiratory system as well as other organs and it can lead to death if left untreated. It is highly contagious and latently persists in over a billion individuals worldwide.

Rapid and more accurate diagnosis is crucial to facilitate early treatment of infectious cases and thus have a substantial impact on tuberculosis control activities.

Objectives: Designed to evaluate the performance of BACTEC MGIT 960 System and compared with BACTEC 9000 MB System and Lowenstein-Jensen (LJ) Solid medium for recovery rate, time to detection and contamination rate.

Methods: 163 clinical samples were processed and inoculated into BACTEC MGIT 960, BACTEC 9000 MB, and Solid LJ medium in Medical Research Council Laboratories, Tuberculosis Diagnostic Laboratory, The Gambia

Findings: BACTEC MGIT 960 detected 87(54.0%), BACTEC 9000 MB 90(55.2%), and Solid LJ medium 67(41.4%) specimens with *Mycobacterium tuberculosis* complex (MTBC). BACTEC MGIT 960 had the shortest mean number of days (10.3) to detection, followed by BACTEC 9000 MB (13.4) and Solid L J medium (26.7). Sign rank test showed all three methods had significant difference in days to detection (each $P<0.0001$). About 39% of detection by BACTEC MGIT 960 took place within the same week, compared to 26.7% and 7.5% by BACTEC 9000 MB and Solid LJ medium respectively. The best yield was obtained with BACTEC 9000 MB System, but when compared with the BACTEC MGIT 960 System, it was not statistically significant. Performances were the same when the combination of a liquid plus a Solid LJ medium were measured ($P=0.05$). Contamination rates were higher in BACTEC MGIT 960 (12.9%). Significantly higher than that of BACTEC 9000 MB System ($P=0.041$) and Solid L J medium ($P=0.022$). BACTEC 9000 MB System and Solid L J medium have similar contamination rates (6.7% and 4.9% respectively; $P=0.607$).

Conclusions: BACTEC MGIT 960 had a shorter time to detection of MTBC than BACTEC 9000 MB and Solid LJ medium. Despite higher contamination rate, its performance did not appear to be inferior.

Keywords: Mycobacterium tuberculosis complex, BACTEC 9000 MB, BACTEC MGIT 960.

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Sensitive and Rapid Tuberculosis Culture Diagnosis with Disposable Filters Replacing the Laboratory Centrifuge

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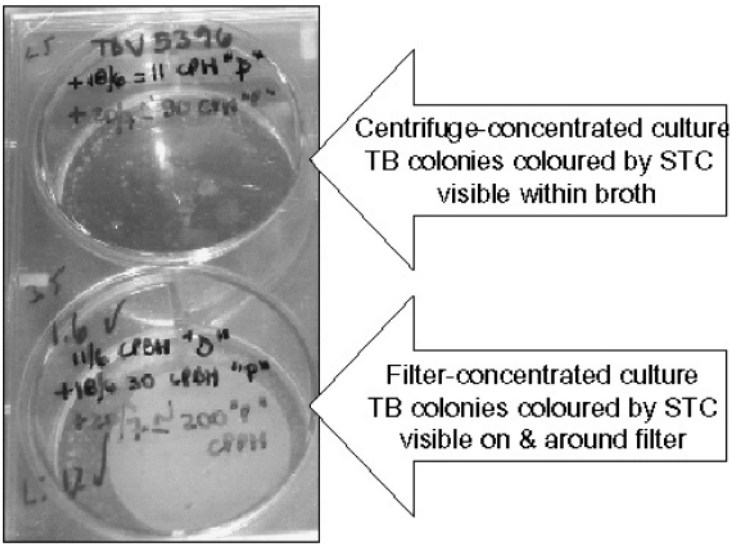
Background: Sensitive culture diagnosis of tuberculosis usually requires centrifugation to concentrate mycobacteria from the decontamination process. Centrifuges are expensive and unreliable and a barrier to the provision of sensitive diagnosis in the field settings where it is most needed. Filters may be used to concentrate tuberculosis and may be an alternative to centrifugation.

Objective: To evaluate filters for replacing centrifugation for tuberculosis culture diagnosis.



Method: Sputum samples that were microscopy-positive for acid-fast bacilli were decontaminated with the conventional n-acetyl cysteine NaOH technique for 20 minutes and addition of excess buffer to 14 ml volume. Half was processed conventionally by 3 000g centrifugation for 20 minutes at 17ºC and inoculation of the product into culture broth. The other half of the sample was aspirated with a 5ml syringe through a disposable 0.4 μm polycarbonate filter that was then placed directly into culture broth. The broth was identical for centrifugation and filter concentration: Middlebrook 7H9 with selectatabs, OADC and 50 μg/ml of the indicator STC (2,3-diphenyl-5-(2-thienyl) tetrazolium chloride). Cultures were examined 3x weekly and colony counts and days to positivity were determined with an inverted light microscope and days to colourimetric positivity by naked eye.

Results: Of the 34 samples, one was culture negative (collected during TB therapy) and another was contaminated in all tests. Analysis focused on the remaining 32 samples, which yielded one false-negative and one contaminated culture by each technique. Centrifuge and filter concentration therefore both had equal 94% (30/32) sensitivity. There were no significant differences between time to diagnosis for centrifuge vs. filter concentration (median 10 vs. 11 days by microscopy, P=0.4; 12 vs. 14 days by naked eye respectively, P=0.3). Naked eye colorimetric detection of TB was significantly slower than microscopic examination (median days delay 2.0 IQR 0-3 range 0-5, P=0.002). There were no significant differences in the numbers of colony forming units between cultures derived from centrifuge vs. filter concentrated sputa (mean 849 SD 555 vs. 920 SD 647 colonies, respectively, P=0.3).

Conclusions: Disposable filters may have the potential to replace centrifugation, maintaining rapid and high-sensitivity TB culture without the expense of centrifuge purchase and maintenance. Current research is evaluating filtration for microscopy and for concurrent tuberculosis diagnosis and drug-susceptibility testing.

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Is There Still a Place for Conventional Methods in the Rapid Detection of Rifampin and Isoniazid Resistance in M. Tuberculosis? The Case of Thin Layer Agar Direct Method

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Tuberculosis is a global problem affecting the under-developed world. Drug-resistant strains and the recent description of XDR strains elicit a need for rapid and low cost drug susceptibility tests (DST).

Objective: evaluate thin layer agar (TLA) for *M. tuberculosis* and resistance to rifampin (RIF) and isoniazid (INH) detection as direct method in patients with MDRTB risk.

Method: 100 adult patients with pulmonary tuberculosis and epidemiological risk for MDRTB were studied. Sputum specimens were NaOH-Nalc decontaminated and auramine-rodamine was performed. Sediment was inoculated in LJ, MGIT® and TLA. TLA plates were divided in quadrants with 7H10 agar media, one as growth control, one with paranitrobenzoic acid (PNB) specific inhibitor of *M. tuberculosis* complex for detection and identification purposes, one with INH 0.2μg/ml and one with RIF 1μg/ml. LJ, MGIT and TLA positivity as well as detection time were compared. Sensitivity (S), specificity (Sp) and predictive values (PV) for RIF and INH resistance in TLA were calculated as compared to conventional indirect DST in 7H10 middlebrook agar, TLA time for resistance detection was recorded and cost were calculated.

Results: MGIT and TLA were more sensitive for detecting *M. tuberculosis* (96.7% and 90.3%) than LJ (84.7%). Contamination rate was 9.7%, 3.2% and 1.1% for LJ, TLA and MGIT. Median time for detection was 22, 10 and 7.6 days for LJ, TLA and MGIT. TLA S, Sp and PV for RIF and INH resistance detection were 100%. TLA mean time for RIF and INH resistance detection was 11 and 11.5 days. The cost to use TLA as screening tests in MDRTB high risk population was one third of the cost of using regular approaches to detect MDRTB patients.

Conclusion: TLA showed a rapid turnaround time for detection and identification of *M. tuberculosis.* In addition, it gives more rapid results and comparable performance to conventional DST methods for detecting RIF and INH resistance. Its simple format makes it a low cost alternative for detecting MDRTB patients in low-resources settings.

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Monitoring Anti-tuberculosis Therapy with Fluorescein Diacetate (FDA) Microscopy Rapidly Determines Infectiousness and Screens for Drug Resistance

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Background: Tuberculosis treatment and infection control are hampered by difficulty assessing mycobacterial viability to determine infectiousness and early treatment response. TB culture takes weeks; molecular tests are technically demanding; and acid-fast staining cannot differentiate live from dead tuberculosis.

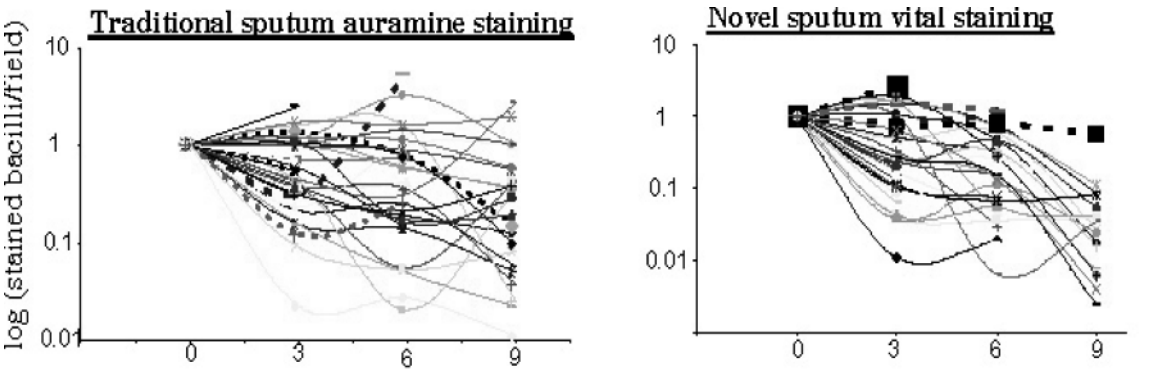
Objective: To develop a simple slide microscopy test to rapidly determine tuberculosis viability.

Methods: A protocol was optimised to stain viable but not dead tuberculosis in decontaminated sputum dried on to microscope slides and stained with fluorescein diacetate (FDA). The reliability of this FDA slide microscopy for determining the concentration of viable tuberculosis in sputum was then compared with quantitative culture.

Results – laboratory evaluation: In untreated patients, tuberculosis auramine staining was unaffected whether sputum was fresh or had been sterilised by boiling, whereas FDA stained only un-boiled, viable tuberculosis. Quantification of viable tuberculosis by culture was reliably predicted by FDA but not by auramine microscopy.

Results – clinical evaluation: Sequential sputums were collected from 35 patients before and after three, six and nine days of first-line tuberculosis treatment. Culture quantification of viable mycobacteria in sputum was predicted by slide microscopy with FDA (r2=0.77) but not auramine (r2=0.33). Quantification of viable tuberculosis in sputum by both quantitative culture and FDA microscopy fell 10-100-fold during the first nine days of treatment in all patients with drug-susceptible tuberculosis, whereas there was little change for patients with MDRTB. Specifically, 70% of samples from patients with drug-susceptible tuberculosis had a decline in the FDA count of viable tuberculosis of at least 0.2 logs/treatment-day, compared with none of the samples from MDRTB patients (P<0.001).

Conclusion: FDA slide microscopy determined the viability of tuberculosis in sputum in minutes, compared with ~1 month required for culture. This simple and inexpensive technique rapidly assessed patient infectiousness on treatment, potentially guiding infection control measures. FDA staining also revealed differences in early treatment response between non-MDR and MDRTB and may allow early field screening for MDRTB and impending treatment failure.



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Comparative Evaluation of Mycobacteriophage Assay and Automated MGIT-960 Culture Method with a Novel ESAT-6 PCR Method for the Diagnosis of Tuberculosis

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Rapid and accurate diagnosis of symptomatic patients of pulmonary tuberculosis is highly desirable not only for instituting adequate treatment but also for minimising the spread of the disease in the community. The conventional methods such as AFB staining and LJ culture methods are either less sensitive and or very time consuming. In this direction automation and molecular techniques have emerged as methods of choice in several labs. However, from India, harboring arguably the largest number of tuberculosis patients, no data is available on the comparative efficacy of various conventional and emerging diagnostic tools. In the present study, we have evaluated five different lab methods for specific and relatively early diagnosis of **Mycobacterium tuberculosis.**

The sputa from 125 patients with presumptive diagnosis of pulmonary tuberculosis and 60 control subjects with no clinical evidence of tuberculosis were subjected to the Acid-fast staining and cultured on the Lowenstein-Jensen (LJ) slants and in automated BACTEC-MGIT-960® system. In addition, newly marketed mycobacteriophage infectivity assay (FASTPlaque® assay) as well as genus and species-specific polymerase chain reaction (PCR) assays were also carried out on these samples simultaneously.

Of the five diagnostic methods, PCR successfully detected all the cases of pulmonary tuberculosis; it was also the most rapid and sensitive method for the specific diagnosis of tuberculosis. On the other hand, the sensitivity of MGIT-960 was found to be 65.5%, followed by FAST Plaque assay (51.4%), LJ culture method (44.28%) and microscopy (40%). The mean detection time for smear positive and smear negative samples was 12.5 and 14 days, respectively by MGIT-960 system and 18 and 25 days, respectively, by LJ culture method. The FASTPlaque failed to detect mycobacteria from the smear negative samples, and therefore had no additional sensitivity. The contamination rates for LJ, MGIT-960 and FASTPlaque assays were 13.3%, 16.6% and 23%, respectively. Out of 70 cases, 46 (65.71%) could be diagnosed correctly by one or more non-molecular techniques while PCR detected additional 11(15%) cases. Therefore, it could be concluded that a mycobacteriology laboratory needs to be equipped with a rapid culture system and PCR facilities, besides basic facilities, to achieve highest possible accuracy in mycobacterial disease diagnosis.

In another study to find the prevalence of extensively drug-resistant tuberculosis (XDR-TB) in Indian AIDS patients, M. tuberculosis was isolated from 24 of 54 AIDS patients seen during March to December 2006. Twelve (50%) of these had resistance to first line drugs while four (33.33%) were extensively resistant to second line drugs tested. All four patients died within 2.6 months of diagnosis.

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Comparative Analysis of Staining Methods for Mycobacterium Species

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Background: Proper diagnosis of M. tuberculosis (MTB) is critical in the fight against tuberculosis (TB) especially in under-developed countries. Diagnostic methods currently used to detect MTB in these countries are limited and most of the available better tests are very costly, require sophisticated equipment mainly available for research purposes only. There has not been widespread implementation of the fluorescent microscopy in most clinics and some hospitals. It is important that available diagnostic tests be optimised and introduced into these communities, and that newer more affordable tests be developed. Lack of timely and accurate diagnosis of TB and drug susceptibility results has contributed to the emergence of drug-resistant TB, contributing to the spread of multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB. The latter is associated with high mortality especially in HIV infected patients. The development and introduction of improved readily affordable and efficient diagnostic test methods would not only help prevent the spread of MTB especially the MDR and XDR TB strains but also guide appropriate treatment for infected individuals.

Objectives: We sought to optimise existing fluorescence stains for acid fast bacillus staining (AFB) of tissue and explore TB-specific ribosomal RNA (rRNA) probes to enhance TB diagnostics through the provision of rapid, easy and affordable methods.

Methods: Formalin fixed paraffin-embedded lung tissue sections from C57BL/6 (B6) and the highly susceptible gamma interferon gene-disrupted (GKO) C57BL/6 mice, which had been infected with low dose aerosol of the Erdman MTB strain, were used for this comparative analysis. Human gut tissue sections containing gram positive and gram negative bowel flora were used as a control. These tissues were used to optimise acid fast staining using the standard Ziehl Neelsen (ZN) and Auramine-Rhodamine (AR) fluorescent stain for AFB. The microscopic performance of both methods was compared. Fluorescent in Situ Hybridisation (FISH) targeting the ribosomal RNA using previously published probes was done on the tissue sections and other cultured isolates of a variety of mycobacteria species to determine the accuracy with which FISH could discriminate between species and, gram positive and negative bacteria based on ribosomal RNA signature sequence. FISH results were scored and compared to the ZN and AR cell wall stains using fluorescent microscopy.

Results: Tissue is an ideal sample to detect AFB positive mycobacteria using fluorescent staining. The optimal conditions for AR staining method for tissues will be presented. Applying these methods to lungs harvested from GKO and B6 mice infected with 50 to 100 CFU Erdman resulted in 7.93Log and 4.89Log CFU respectively 28 days after infection. We found that the AR method was more sensitive, easier to interpret and less time-consuming than traditional ZN method. It resulted in less eye strain than the ZN method. Interestingly, a staining difference was noted between GKO and the C57BL/6 using AR. The GKO-infected animal showed a mixed population of red and green bacilli whereas the B6 mice showed the characteristic red/orange staining. Our results suggest that the physiologic state of the organisms based on the immune status of the host differs. FISH was as sensitive as AR in detecting AFB positive organisms and was species-specific based on our finding that it accurately discriminated between mycobacterium species.

Conclusion: Introduction of fluorescence microscopy as a means of diagnosing MTB would provide cheap, efficient and timely diagnosis of MTB. Due to recent availability of low cost fluorescent microscopes (e.g. LED light sources), it is possible that these microscopes could also be used in rural areas where electricity is sporadically available. Use of this technology could accommodate AR and FISH methods. Integration of some of our methods would also potentially improve the timely diagnosis and treatment of the people infected with MTB. Future work will focus on direct detection of drug resistance using FISH and furthermore the AR stain may yield important insights regarding cell wall characteristics of MTB in immunocompetent and immune compromised hosts.

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Cord Formation: A Good Tool for Presumptive Identification of M.tuberculosis Complex

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Background: For TB patients co-infected with HIV, the rapid differentiation between tuberculosis and non-tuberculous mycobacteria (MNT) is fundamental. The medical management of patients afflicted by mycobacterial infections, including decisions related to administering specific treatment and patient isolation, depend on the rapid and precise identification of the mycobacteria involved. This often represents a substantive challenge for the diagnostic laboratory. The identification of Mycobacterium tuberculosis complex (MT), using non-molecular methods, is time-consuming. Some studies have evaluated the utility of cord formation in liquid or solid medium for the presumptive identification of MT and to guide decisions regarding susceptibility testing or species identification. The distinctive colony morphology of mycobacteria when grown on solid medium also helps to characterise the species. Our laboratory has extensive experience using a screening test which consists of visual analysis of colony morphology on solid media and the presence of cording on microscopy for presumptive identification of MT.

Figure 1

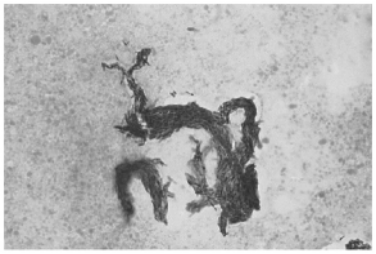
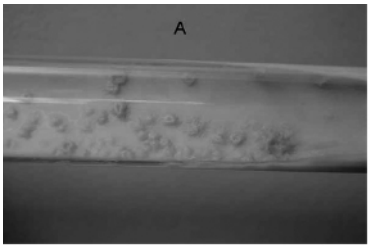


Figure 2



Objective: To evaluate the cord formation test in order to introduce it as a screening method, while taking into account the cost and the time it takes to get the result.

Methods: A total of 152 strains were tested by the screening test consisting of microscopy examination (smear stained by Ziehl-Neelsen) (figure 1) and the culture macroscopic observation (figure 2). A molecular method was used as gold standard to ascertain the confirmed identification result. The molecular method, PCR restriction enzyme analysis (PRA) consists in the amplification of a 441-bp fragment from the hsp65 gene by polymerase chain reaction (PCR). A comparison was made concerning the costs and time of both methods. Costs were estimated by including the price of all the material needed for each test to be conducted. The time was estimated by the evaluation of time spent from the beginning of the test until the conclusion of the result.

Results: There was disagreement between the two methods in only one strain (0.6%). In the six cases where there was a preliminary disagreement between the cord formation test and the PRA, the evaluation of the macroscopic aspect resulted in agreement with the gold standard. The co-positivity of the screening test was 100% and the co-negativity was 98%. The cord formation costs US$ 0.25 whereas the PRA test costs US$ 7.00. The result of the cord formation test is ready in two days, whereas the PRA test needs four days.

Conclusions: The presumptive identification of MT using macroscopic analysis of colony morphology associated with the presence of cording on microscopy is a simple, rapid and low-cost test. The results showed that this method can be introduced in the laboratory network as presumptive method in the tuberculosis diagnosis, before sending the culture to a reference centre for confirmatory tests.

Aknowledgement: Work funded by the ICOHRTA AIDS/TB project, FIC/NIH # 5 U2R TW006883-02.
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Direct Detection of Rifampin Resistance in Mycobacterium Tuberculosis by the Nitrate Reductase Assay Applied Directly in Sputum Samples

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Background: Several factors are responsible for the resurgence of tuberculosis (TB) worldwide. Inadequate treatment favours the development of multidrug-resistant tuberculosis (MDRTB). A cost-effective and rapid drug susceptibility testing (DST) method is required to guide TB treatment. Conventional methods for DST of M. tuberculosis require several weeks to give results. Commercially available systems such as BACTEC MGIT 960 and MB/BacT are faster but demand costly equipment and supplies, and are therefore not feasible in most resource-poor settings. Resistance to rifampin (RIF) is an important predictor for the early diagnosis of MDRTB.

Objective: To compare the nitrate reductase assay (NRA) with the proportion method (PM), considered as gold standard, to detect RIF resistance in M. tuberculosis directly from sputum samples.

Methods: The study was carried out by four regional laboratories from the state of São Paulo, Brazil. A total of 206 sputum samples tested smear positive from patients with pulmonary tuberculosis. The sputum was decontaminated by the Petroff method and DST to RIF was carried out using the PM and the NRA.

Findings: The results of the DST obtained directly from sputum were: six samples resistant to RIF (figure 1) and 200 samples susceptible (figure 2). No discordance was observed between the two methods. The sensitivity and specificity of the NRA was 100%. Results were available in 10 days for 66 (34%) samples, 15 days for 102 (53%) samples and 20 days for 24 (13%) samples. The results of PM took 30 days to be available. Conclusions: the NRA proved to be a promising method for the screening of suspect MDRTB cases directly from sputum samples. The simplicity of the method, its low cost and celerity to give the results make it a good alternative method for laboratories in resource-poor settings.

Figure 1

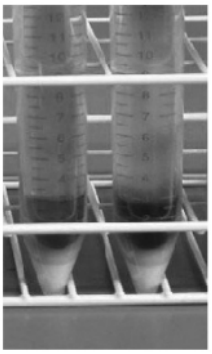
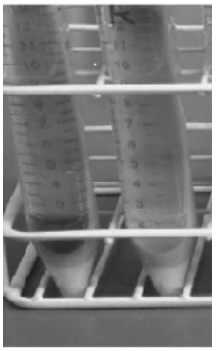


Figure 2



Acknowledgement: this study was partially funded by INCO-Dev ICA4-CT-2001-10087. Contact: Maria Alice da Silva Telles, Setor de Micobactérias Instituto Adolfo Lutz, Av. Dr. Arnaldo, 355 São Paulo-SP 01246-902 – Brazil. atelles@ial.sp.gov.br; atelles@osite.com.br

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Expedited Smear Microscopy Approach for the Diagnosis of Tuberculosis

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Background: The diagnosis of tuberculosis in most resource-limited countries relies on smear microscopy. Three sputum samples are required for examination and at least two should be positive to declare smear positive TB. Due to the requirement for repeated health facility visits, some patients drop out from the diagnostic process. Peripheral laboratories are overburdened and resources are scarce in countries where TB is very common. Optimising smear microscopy for the diagnosis of TB is urgently required.

Objective: To compare the yield of smears collected in an accelerated scheme (one sample collected one hour after the first spot smear) and the standard spot-morning-spot approach.

Methods: Four studies using similar study design enrolled consecutive patients having a cough for ≥ 3 weeks attending health facilities in Ethiopia, Nigeria, Nepal and Yemen. Patients submitted three sputum samples as spot-morning-spot (the routine standard screening process). An additional sample (extra-spot) was collected one hour after the first spot. All smears were examined using hot Ziehl-Neelsen smear microscopy. The yield from the standard (spot-morning-spot) and the accelerated (spot-extra spot-morning) approaches were compared.

Results: A total of 923 patients were recruited from the four countries and 216 (23%) had at least one positive smear. The first-spot samples detected 181 (84%) of these patients. The morning sample detected an additional 22 (10%) and the second-spot 7 (3%) patients with a cumulative yield of 210/216 (97%).The extra-spot detected 18 (8%) additional patients missed by the first-spot and the morning detected 11 (5%) patients missed by the first and extra spot samples, with a cumulative yield of 210/216 (97%). The first day smears detected 84% (181/216) patients using the standard approach and 93% (200/216) by the accelerated approach. There were no significant differences across countries although sample sizes are small to detect differences.

Conclusion: Results from the four countries indicate that the first two specimens collected the day of the first visit can identify most patients with positive smears. Effectiveness studies in programmatic settings of the feasibility in implementing these changes on a larger scale are needed.

Table: Incremental yield of serial smears using the standard and accelerated sputum collection approaches

Country	No	≥ 1 pos. smear N (%)	1st spot Pos. N (%)	Incremental yield of standard approach		Incremental yield of accelerated approach		Total detected by two-day smears		N identified by the first day smears	
				Morning N (%)	2nd spot	Extra-spot	morning	Standard approach	Accelerated approach	Standard approach	Accelerated approach
Ethiopia	243	52 (21)	45 (87)	4 (8)	2 (4)	4 (8)	3 (6)	51(98)	52 (100)	45 (87)	49 (94)
Nigeria	224	48 (21)	45 (94)	3 (6)	0	2 (4)	1 (2)	48 (100)	48 (100)	45 (94)	48 (100)
Yemen	250	61 (24)	52 (85)	8 (13)	0	7 (12)	1 (2)	60 (98)	60 (98)	52 (85)	59 (97)
Nepal	206	55 (25)	39 (71)	7 (13)	5 (9)	5 (9)	6 (11)	51 (93)	50 (91)	39 (71)	44 (80)
All	923	216 (23)	181 (84)	22 (10)	7 (3)	18 (8)	11 (5)	210 (97)	210 (97)	181 (84)	200 (93)

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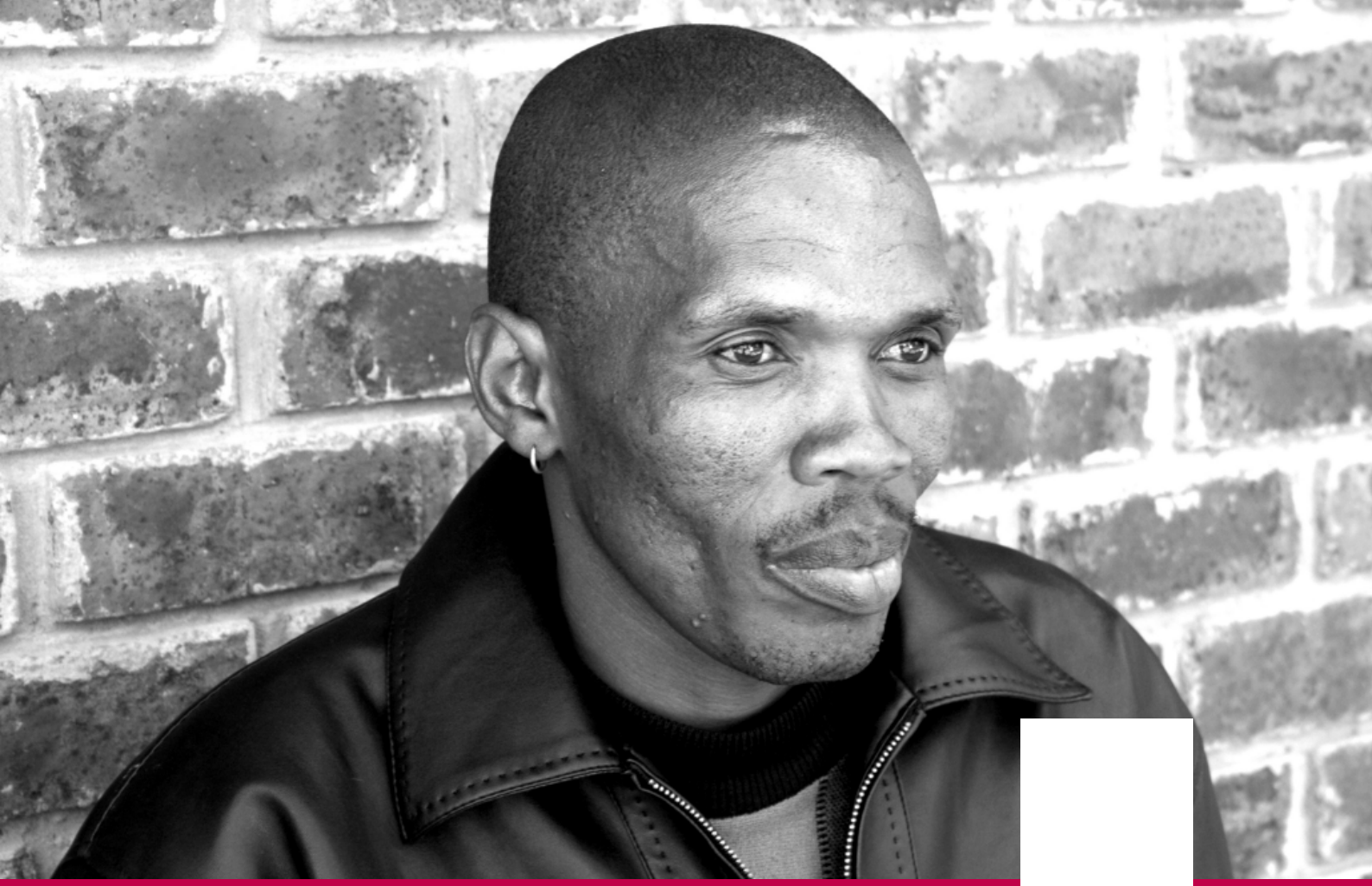

Joseph Ramokoatsi, HIV/ TB counselor and patient in rural Lesotho

TB DYING FOR A TEST

Session 2

Operational Challenges for Field Diagnostics

Challenges in TB Diagnostics in Secondary and Local Government Health Institutions in Ibadan, Nigeria

Bukola O. Ayinde[1], Obatunde O. Oladapo[2]

Background: Improved public health, BCG vaccination, introduction of anti-tuberculosis drugs and Isoniazide preventive therapy has been known to reduce the incidence of TB (tuberculosis) disease over the years. The global HIV epidemic has led to a resurgence of TB diseases, which remain a serious threat, especially in people infected with HIV. Worldwide, TB is responsible for death in one-third of people living with HIV/AIDS (PLWHA) and is the leading cause of death among PLWHA. There is a need for comprehensive TB treatment for PLWHA. A good TB control programme will include comprehensive case studies of patients suspected of having TB; prompt and effective TB diagnosis and readily available and proper treatment.

Prompt and effective TB diagnosis is crucial to TB treatment as it serves as the basis and indication for treatment. The emergence of MDR-TB and XDR-TB threatens TB control efforts worldwide. To reduce the time needed for diagnosis, new tools for rapid TB diagnostics testing are urgently needed. In order to promote progress in TB diagnostics, there is a need to identify and address those factors hindering TB diagnostics in resource-poor settings at various levels in the health care system.

Objective: To identify limitations to the available TB diagnostics methods and highlight obstacles to rapid and effective TB diagnostics from the perspective of health care providers in two secondary health institutions and two local government health care centres in Ibadan, in Oyo State, Nigeria.

Method: In-depth and key-informant interviews were carried out among selected relevant health care providers at TB centres in two secondary health institutions and two local government health centres located in Ibadan city. These include five doctors working in TB treatment centres, 10 nurses in the TB units and five laboratory scientists engaged in TB diagnostics work.

Findings:

Limitations of available diagnostics tools

The following limitations were identified by the respondents about available TB diagnostic tests (tuberculin skin and sputum smear) in Nigeria:

- They are not rapid and accurate
- They have low sensitivity
- They cannot differentiate between latent TB infection and active TB diseases
- They cannot detect MDR-TB and XDR-TB
- They take 2–3 days to produce a result
- The tests cannot detect TB infection in new-born babies
- False results are sometimes obtained
- People who are co-infected with HIV infection and TB disease may not react to tuberculin skin test
- For some patients, a combination of tuberculin, sputum smear and X-ray is required for accurate diagnosis
- Administration of the tuberculin skin test depends on expertise of personnel. If it is not carried out properly, the results may not be accurate
- The tuberculin test requires that patients come back for response reading on the arm. Failure of patients to return at the appropriate time nullifies the results
- For HIV-positive patients who cannot produce sputum, diagnostics may be limited to X-ray and symptoms for diagnosis

Obstacles to rapid and effective TB diagnostics test:

- Irregular supply of electricity hinders prompt diagnostics since microscope and X-ray machines require electricity to function
- Self-medication with some antibiotics may mask TB symptoms or affect laboratory test results
- Failure to make regular hospital appointments will cause delay in proper diagnostic investigation

- Most HIV treatment centres do not have on-site TB diagnostic units. Patients are usually referred and this might delay diagnosis or result in the patient not reporting to the referral centre
- Too few personnel trained in aspects of TB diagnostics
- In poor resource settings, there are no tools for the diagnosis of MDR-TB and XDR-TB. Even in cases where the tools are available, diagnosis is slow, taking between six and 16 weeks and requiring specialised and costly equipment
- Damien Foundation prepares the diagnostic reagent in bulk. As each laboratory has to make a requisition, this sometimes causes delay
- Poor accessibility to and appropriateness of TB diagnostic services
- Poor coordination between sample collection centres and diagnostics centres
- Poor funding services for TB programmes
- Lack of community involvement in TB programmes

Conclusion: From the findings, the limitations and hindrances to effective diagnostics can be broadly summed up as:

- Deficiency of tools, personnel and testing centres, as well as poor funding.
- We can start saving lives now by strengthening health care systems, supporting research and development of new ways to diagnose and treat TB, especially among PLWHA. There is the need to provide rapid, inexpensive diagnostic tests at the point of testing and also to increase the number of health care units with staff trained in TB detection, diagnostics and treatment.
- In view of the numerous limitations and hindrances to the available diagnostic testing methods, the need for research to discover more efficient and rapid TB diagnostic tools has become imperative. New, improved diagnostic methods for detecting TB infection are highly desirable.

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Evaluation of Fluorescence Microscopy for Diagnosis of Pulmonary Tuberculosis in a High HIV Prevalence Setting

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Background: Direct (unconcentrated) smear microscopy (DM) is the primary diagnostic test for TB in low income countries and will likely remain so for the foreseeable future. DM is rapid, inexpensive, and highly specific for TB in high incidence settings. However, DM has poor overall sensitivity (average < 60%) and sensitivity is further decreased in the setting of HIV co-infection. Methods to improve smear microscopy have therefore been identified as a major priority for global TB control efforts. Fluorescence microscopy (FM) has replaced DM as the standard initial diagnostic test for TB in high income countries. Advantages of FM include the simplicity of the staining method and the decreased time required to read results. FM has also been reported to have increased sensitivity and similar specificity compared with DM. However, there are limited data evaluating FM in the setting of HIV co-infection and previous studies have mostly been conducted in university or research centres rather than in local settings. To address these issues, we report preliminary results from a prospective evaluation of FM performed within a national TB programme in sub-Saharan Africa.

Objectives: To compare the sensitivity and specificity of DM and FM performed at the Uganda National Tuberculosis and Leprosy Control Programme (Uganda NTLP) for the diagnosis of pulmonary TB.

Methods: Consecutive patients who were admitted to Mulago Hospial in Kampala, Uganda with respiratory symptoms > 3 weeks, a clinical suspicion of pneumonia, and who were not already receiving anti-TB treatment were enrolled. All patients underwent HIV testing; those who were HIV seropositive underwent CD4+ T-lymphocyte count determination. For each patient, two sputum specimens (random and early morning) were submitted for DM, FM, and mycobacterial culture. Patients were included in the present study if smear microscopy and culture results were available from both sputum specimens. DM was performed using the standard Ziehl-Neelsen staining method and FM was performed on concentrated (NALC-NaOH method) specimens using the auramine-O staining method. HIV-infected patients with negative sputum smear results were additionally evaluated by bronchoscopy with broncho-alveolar lavage (BAL). Any positive mycobacterial culture of sputum or BAL specimens performed on Lowenstein-Jensen medium was used as the gold standard for TB diagnosis.

Table 1. Sensitivity and Specificity of Individual Sputum Specimens

Sensitivity	Sputum 1 (N=25 Culture +)	Sputum 2 (N=27 Culture +)
DM	14/25 (56%)	19/27 (70.4%)
FM	19/25 (76%)	21/27 (77.8%)
Specificity	Sputum 1 (N=44 Culture -)	Sputum 2 (N=42 Culture -)
DM	42/44 (95.5%)	40/42 (95.2%)
FM	41/44 (93.2%)	39/42 (93%)

Table 2. Overall Diagnostic Performance

	DM	FM
Sensitivity (%)	63	75*
Specificity (%)	95	92
PPV (%)	91	89
NPV (%)	75	81
* p<0.04 for comparison of DM and FM		

Results: Between April and June 2007, there were 69 patients meeting study criteria. HIV infection was confirmed in 60 (87%) patients who had a median CD4+ T-lymphocyte count of 54.5 cells/mm3 (IQR 14-160). Mycobacterial culture results were positive in 25/69 (36%) and 27/69 (39%) patients for the first and second sputum specimens, respectively. The sensitivity and specificity of DM and FM for each individual sputum specimen are shown in Table 1. All specimens positive by DM were also positive by FM. The sensitivity of FM was 20% higher in the random sputum specimen (Sputum 1) but only 7% higher in the early morning sputum specimen (Sputum 2). When combining results from both sputum specimens and considering BAL culture results, culture-confirmed tuberculosis was diagnosed in 32 (46%) patients. The overall diagnostic performance of FM and DM is shown in Table 2. The

sensitivity (75% vs. 62%, $p<0.05$) and negative predictive (81% vs. 74.5%) of FM were higher than for DM. The specificity and positive predictive value were similar for both DM and FM.

Conclusion: In an HIV-infected cohort with an advanced degree of immunosuppression, FM was 13% more sensitive than DM for the diagnosis of pulmonary TB. Our data are similar to findings from a recent meta-analysis showing that FM was approximately 10% more sensitive than DM in HIV-uninfected persons. The sensitivity of both DM and FM in our study was higher than results typically reported in the literature in HIV-infected populations, probably reflecting the high quality of smear microscopy evaluation at the Uganda NTLP and possibly a higher bacillary burden in our hospitalised cohort. Overall, our preliminary data support the feasibility and value of FM in resource-limited settings with high prevalence of both TB and HIV.

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Body Mass Index is More Reliable Than Tuberculin Skin Testing for Diagnosing Adult Pulmonary Tuberculosis in Endemic Regions

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Background: The tuberculin skin test is often used to assess the likelihood that symptoms are caused by tuberculosis disease, especially in patients who are unable to provide sputum. Clinicians may believe that tuberculosis disease is unlikely if the tuberculin skin test is negative and consider tuberculosis disease particularly likely if the tuberculin skin test is very strongly positive. However, reliability may be limited because patients with untreated tuberculosis may have anergic, false-negative tuberculin skin test results and in tuberculosis-endemic countries many healthy people have positive tuberculin skin tests. Furthermore, tuberculosis is a wasting disease and we hypothesised that low body mass index may be more reliable for diagnosing tuberculosis disease than the tuberculin skin test.

Objective: To compare the diagnostic utility of the tuberculin skin test vs. body mass index for diagnosing tuberculosis disease in adults in Peru.

Methods and results: In a prospective case-control study we that found that 46% (298/647) of healthy individuals were tuberculin skin test-positive vs. 74% (261/355) of newly-diagnosed, untreated pulmonary tuberculosis patients (odds ratio of tuberculosis if the tuberculin skin test was positive 3.4, 95% confidence intervals 2.6–4.6, $p<0.001$). The size of the tuberculin skin test reaction was not associated with the likelihood of tuberculosis, even for large reactions >20 mm. Neither tuberculin skin test size nor positivity were associated with the patient prognosis during treatment or two years follow-up (odds ratio of good prognosis 0.8, 95% confidence intervals 0.35–1.8, $p=0.6$). Low body mass index was associated with tuberculosis disease and with false-negative tuberculin skin test. Consequently, body mass index <20 kg/m2 predicted tuberculosis disease more reliably than the tuberculin skin test result (odds ratio of tuberculosis if body mass index <20 kg/m2 was 7.7, 95% confidence intervals 5.2 – 11.4, $p<0.001$).

Tuberculosis disease remained only weakly associated with the tuberculin skin test result and more strongly associated with body mass index if HIV-positivity was adjusted for in the analysis, or if the HIV-positive patients were excluded. HIV infection is uncommon in Peru, affecting approximately 5% of tuberculosis patients, so to better characterise the effect of HIV infection an additional group of HIV-positive newly-diagnosed tuberculosis patients also had tuberculin skin tests and 68/75 (91%) had false-negative results.

Literature review revealed that these findings for tuberculin skin test results in HIV-positive and HIV-negative newly-diagnosed tuberculosis patients and in healthy people were consistent with case-series data from other endemic regions. The data are confused by the fact that almost all tuberculosis patients during or after treatment are tuberculin skin test positive, but false-negative test results are common at the time of diagnosis.

Conclusions: In tuberculosis high-burden countries, approximately one half of healthy adults and only approximately three quarters of newly-diagnosed tuberculosis patients have positive tuberculin skin tests, limiting the diagnostic utility of this test, even for strongly positive results and especially in HIV-positive people. Low body mass index was a better diagnostic indicator of active tuberculosis than a positive tuberculin skin test. Therefore, in adults from endemic regions, the likelihood that an illness is caused by tuberculosis is influenced little by the tuberculin skin test result and not at all by the size of positive reactions, but greatly by the body mass index. The frequent policy of using the tuberculin skin test positivity or size to assess whether a patient's symptoms are caused by tuberculosis has little value in adults in endemic regions. Instead, body mass index should be given greater emphasis in assessing the likelihood that a patient's illness is tuberculosis.

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Multi-Drug Resistant Mycobacterium Tuberculosis (MDR-TB) in Ibadan, Nigeria: Challenges and Prospects

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Background: Information on drug resistance to M. tuberculosis in Nigeria is largely unavailable despite the fact that the country ranks 4th in the World Health Organisation's designated 22 countries with a high burden of TB. The data obtained will be useful to strengthen TB control programmes in Nigeria.

Objectives:
(1) To determine the prevalence of MDR-TB among new cases of pulmonary TB (PTB) in Nigeria.
(2) To create a baseline data for the development of intervention programmes against MDR-TB in the country.

Materials and Methods: This prospective study was carried out at University College Hospital (UCH), Ibadan, Nigeria. The TB laboratory at UCH, Ibadan, is a designated reference centre with facilities for isolation of Mycobacterium tuberculosis. Specimens are received from medical outpatient clinics including an antiretroviral clinic, neighbouring hospitals and from distant peripheral centres.

Specimens from clinically confirmed new PTB patients from within and outside UCH that were sent to the TB laboratory between 1 May 2005 and 27 April 2006 were recruited into the study. Demographic information about the patients who submitted the specimens was extracted from the accompanying request forms.

Three specimens (labelled a, b and c) from each patient were collected in well-labelled, wide-mouth containers covered with lids. They were transported to the laboratory for processing. Specimens containing saliva were discarded. Each specimen was smeared, air-dried, fixed and stained with Ziehl-Neelsen (Z-N) reagents using a known acid fast-bacilli (AFB) stained slide as positive control and a stained slide made of egg albumin as negative control. Results were recorded according to the grading system of the International Union against Tuberculosis and Lung Diseases as –, scanty, +, ++, or +++ AFB. Then one of the specimens was cultured onto Lowenstein-Jensen (LJ) slope incubated at 37oC for six to eight weeks.

Mycobacterium tuberculosis strain H37RV and sterile LJ medium were used as positive and negative controls respectively. Growth on LJ medium was re-stained with Z-N reagents at two, four, six and eight weeks of incubation .Thereafter, visible growth was confirmed as M. tuberculosis by standard biochemical methods. Confirmed isolates were collated and stored at 4oC in the refrigerator while contaminated cultures were noted and discarded.

Revived M. tuberculosis isolates on LJ slope were sent for drug susceptibility testing against streptomycin, ethambutol, isoniazid and rifampicin using semi-automated radiometric method (Bactec 460 TB) at the Nigerian Institute for Pharmaceutical Research and Development, Abuja, which enjoys support from the National Institute of Health, United States of America. Patients from whom MDR-TB was isolated were referred to the attending physician for further evaluation and treatment.

Findings: Of the 1120 sputum samples that were processed during the study period, 80 (7.1%) were AFB positive while a lower percentage (5.0%) were culture positive even though the association was not statistically significant ($p>0.05$). Sixteen (1.4%) were AFB negative but positive for culture while eight (0.7%) were screened AFB positive but culture negative. Only 40 (3.6%) were AFB and culture positive while the majority, 1 056 (94.3%), were negative for the two tests. Culture contamination rate was found to be 8.8%.

Furthermore, 30 (53.6%) of the 56 culture positive isolates were resistant to both isoniazid and rifampicin; 16 (28.9%) to all the four drugs tested, while 26 (46.4%) were susceptible. Of the 30 resistant isolates, 16 (53.3%) were from the antiretroviral clinic, four (13.3%) from medical outpatient clinic and 10 (33.4%) were from distant health centres.

Conclusion: MDR-TB is emerging in Nigeria. There is a need to strengthen DOTS strategy in Nigeria through expansion of laboratory capacity to carry out quality-assured microscopy, culture and drug susceptibility to reduce TB burden in the country. Also, incorporating active case findings for TB among HIV/AIDS patients will help to reduce the burden of the two diseases in Nigeria.

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Diagnosing Abdominal Tuberculosis: A Retrospective Study from Nepal

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Introduction: According to the World Health Organisation (WHO), nearly one-third of the world's population is at risk of acquiring tuberculosis (TB) and more than 30 million deaths occu rred due to TB in the nineties especially in Africa and Asia.[1] Nepal is a high-burden country for TB with an estimated 6 000 – 8 000 deaths annually. [3] TB can affect any part of the body and abdominal TB accounts for nearly 2% of the total cases of TB.[4]

The clinical presentation of abdominal TB is non-specific and requires confirmatory evidence in order to issue accurate diagnosis.6 A study from the UK reported that laparoscopy may be an investigation of choice among patients from high-burden countries with a suspected abdominal TB clinical history.7 Insidious onset and non-specific clinical and radiological findings of abdominal TB mimics several diseases, such as Crohn's disease, carcinoma, sarcoma, amebiasis, Yersinia sp. infection, gastrointestinal histoplasmosis and peri-appendiceal abscess.9,10 In Nepal, however, though a country with a high incidence of TB, there is a paucity of studies on abdominal TB. During our literature review, we could locate only one study from Nepal regarding abdominal TB 12. The study evaluated the clinical features, diagnostic investigations, surgical treatment and pathology of abdominal TB. This study was conducted three decades back and cannot be extrapolated well to the current settings. 12 We therefore performed a new study with the following objectives:

Objectives:

1. To describe the clinical presentation and the diagnostic and treatment methods of the patients diagnosed with abdominal TB in Nepal.
2. To determine the accuracy of abdominal TB diagnosis in Nepal.

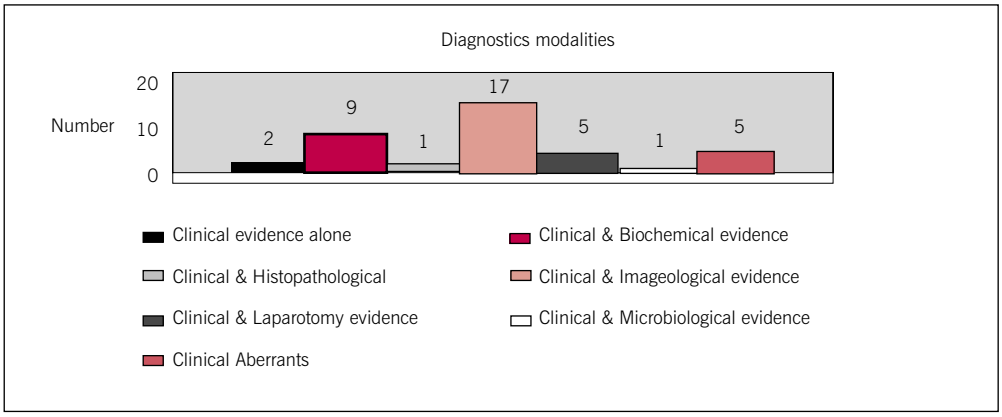
Methods: Altogether, 32 cases of abdominal TB (10.59% of the total extra pulmonary TB) were identified among the total 302 extra pulmonary TB patients at Manipal Teaching Hospital during the period from April 2003 to December 2006. All the cases diagnosed as Abdominal TB were selected for this study. The details of demographic information, clinical presentation, and co morbid conditions were recorded from the medical case files. The laboratory and imageological reports and other necessary information were also obtained and the information was recorded on a proforma designed for the purpose.

Results: From the 32 cases of abdominal TB, 17 (53.13%) were females. The Mean – SD age of the patients was 39.62 – 21.18 years. The common symptoms exhibited by the patients were weight loss [100% (n=32)], loss of appetite [100% (n=32)], fever [71.88% (n=23)]. The personal history revealed smoking in the case of 40.63% (n=13) of the patients and alcohol intake in the case of 37.5% (n=12) of patients. Diagnostic methods in patients with abdominal TB evaluated in our study were divided into seven groups according to the method of diagnosis. Cases that were misdiagnosed and confirmed otherwise later, were also included. The analysis revealed that most of the cases were diagnosed on the basis of clinical presentation along with imageological evidence in our institute. The diagnostic methods used in this study are listed in Figure 1. The treatment was given as per the WHO guidelines with category I [46.88% (n=15)] and category III 53.13% (n=17)] drugs. One patient (3.13%) died due to drug toxicity.

Conclusion: The clinical presentations of abdominal TB appear not specific for the condition. Thus, careful approach and supportive results are required in order to issue the final diagnosis. Measures are to be taken not to misdiagnose other morbid conditions as abdominal TB. If diagnosed early, it can be treated successfully with the conventional anti-TB drugs.

Keywords: Abdominal TB, Diagnosis, Nepal

Figure 1. Diagnostic methods used in this study for diagnosing Abdominal TB



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Investing in People – The Importance of Quality Assurance in TB Diagnostics in Developing Countries

Luc Jansens, MD, DTMH, Nina Sofie LeGrand

Today Sputum Smear Microscopy still remains the optimal diagnostic tool for AFB + pulmonary TB in developing countries. It is fast, relatively accurate and very cost effective. AFB Smear Microscopy may have certain disadvantages compared to QFT, MODS and culture, but one parameter (and potential shortcoming) they all share is the lab technicians' performance. Investing in people thus becomes just as important as investing in research and development for new improved TB tests. We need tests that are as easy (user friendly) to identify AFB negative pulmonary TB patients and MDR/XDR. Whatever the cost, ease of implementation/execution of a test and intrinsic parameters such as sensitivity and specificity, the quality of the execution by well-trained and coached staff will remain the essential factor for the impact of the test result. The more complex the test procedures, the stronger the need for training and coaching. Our experience shows that even the relatively simple implementation of AFB smear microscopy needs very involved and frequent hands-on supervision and on-the-spot training.

The USAID-funded Tuberculosis Treatment and Control Program (TTCP), Georgia has been active in Georgia since 2003 and got involved in quality assurance of seven designated labs in the country in 2005. With minimal expenditure we have been able to improve smear quality by as much as 70% in certain locations and worked out implementation and sustainability plans that could be applied nationwide. Our experience has shown us that there is a direct correlation between laboratory performance and the involvement, expertise and dedication of its quality assurance team. With frequent supervisions we were able to increase percent of acceptable slides from 17 to 97, and positive result reporting accuracy has reached 100% (from as low as 50% at some laboratories). With regular supervision the performance remains high. However, as soon as supervision becomes sporadic, mistakes made by technicians become more numerous.

Our results show that, prior to/along with investing money in expensive equipment and test supplies, we should invest more effort in human resources.

High Level of Discordant Igra Results in HIV-infected Adults and Children

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Objective: To measure the agreement of 2 IGRAs and the tuberculin skin test (TST) for the detection of M. *tb* infection in HIV-infected adults and children and assess the influence of *M.tb* exposure, age and CD4 T lymphocyte count.

Design: Cross-sectional study

Results: In HIV-infected adults (20) and children (23), tests yielded discordant results with 61% (T SPOT.*TB*), 41% (TST) and 28% (QuantiFERON® TB Gold (QTF) of individuals having a positive test. In children, there was poor agreement between the TST and T SPOT.*TB* (Kappa; k=-0.02), but moderate agreement between the TST and the QTF (k=0.33). In adults, there was moderate agreement between the TST and T SPOT.TB (k=0.43), and the TST and QTF (k=0.46). In both children and adults, there was fair agreement between the T SPOT.*TB* and QTF (k= 0.33). The proportion of discordant results ranged from 0 to 39%; 20% of adults had one or more indeterminate IGRA results.

Conclusions: There is poor agreement between the TST and IGRAs in HIV-infected adults and children and age-specific interpretation may be required. Longitudinal assessment is needed to elucidate the impact of low CD4 counts, anergy and the predictive value of IGRAs.

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Setting: Tygerberg district, Western Cape Province, South Africa.

How Dangerous are Tests for Drug-Resistant Tuberculosis?

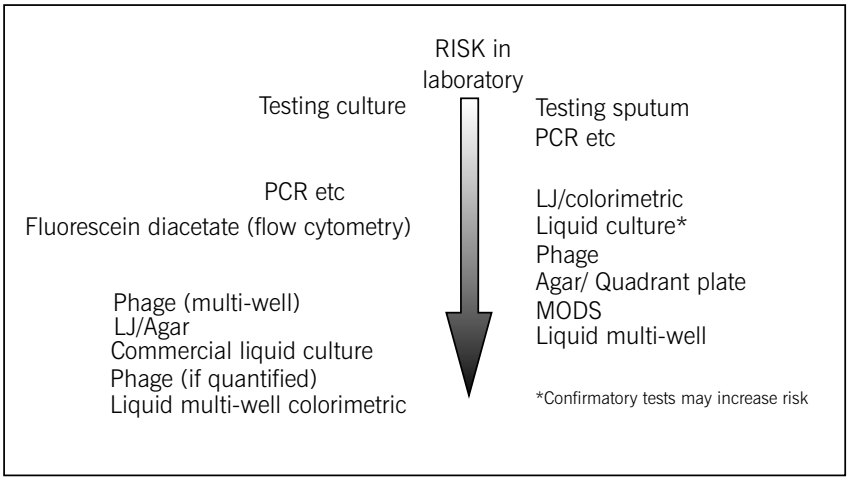
Ruth McNerney

There is an urgent need to strengthen laboratory capacity to screen for MDR-TB. The emergence of XDR-TB has heightened awareness of the need to avoid nosocomial transmission. Numerous technologies are available to assist with the detection of drug- resistant disease, either by screening cultured isolates or through direct testing of clinical specimens.

Study Design: A review of tests for detection of MDR-TB was undertaken. A theoretical risk assessment was undertaken for each of the technologies. Assessment included risks associated with sample collection, sample processing and disposal. Risk to patients, clinical/ nursing staff and laboratory personnel were considered. .

Main Findings: Whereas the rapidity and expense of new laboratory tests is frequently discussed, risks associated with the technology have received less attention. Tests that require repeated handling of infectious materials have intrinsically higher risk of infection for laboratory personnel. Tests that require collection of smear positive sputum specimens may involve increased risk for patients and clinical staff. Molecular tests that do not require viable organisms have intrinsically lower risks than traditional phenotypic methods.

Conclusions: The safety of technology for detecting drug-resistant tuberculosis warrants increased attention. Molecular tests for MDR-TB may be more appropriate than phenotypic methods in low resource settings with poor laboratory infrastructure.



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Estimating the Resource Need for Using Culture to Diagnose Tuberculosis

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Background: The convergence of HIV and tuberculosis has led to a substantial increase in the number of cases of tuberculosis. A large proportion of the HIV-related cases cannot be diagnosed with sputum smear microscopy, the traditional cornerstone of most countries' tuberculosis case-finding programmes. This frequently results in delayed diagnosis and treatment and worse outcomes. Widespread expansion of culture facilities for *Mycobacterium tuberculosis* (MTB) and their use as routine diagnostic measure has been proposed as a means to improve timely case detection and also to provide information about drug susceptibility in order to guide treatment decisions. Such an expansion will require a huge number of additional cultures to be processed. Currently, however, there is a dearth of information about the costs of culture in low-income countries, such as in sub-Saharan Africa, and the scale of resources needed to extend the role of culture in routine programmes.

Objective: This presentation aims at discussing estimates of required resources if culture were to be used for routine diagnosis on a global scale.

Approach: We studied the cost and cost-effectiveness of solid and liquid culture in the Zambian national reference laboratory and translated these costs into international dollars that are transferable to other settings. Costs may still differ from country to country depending, for instance, on prices for consumables from different suppliers or different shipping costs. However, these cost estimates can serve as a basis for calculating the global resource need for the expansion of culture.

Findings: Based on the above approach, we calculated a cost of 91 to 185 international dollars per MTB identified depending on the rate of throughput when using the most cost-effective method for culturing in Zambia (manually read liquid culture). If culture were to be used only to diagnose smear negative TB, costs would rise to 263 to 545 international dollars per MTB identified.

Discussion: We will discuss the implications of these costs in terms of resources likely to be needed when using culture on a global scale with 8.8 million expected cases. These estimates help to inform the debate about expanding the use of culture and the magnitude of resources needed under different policy considerations (e.g. culturing all specimens or only culturing smear negative specimens).

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Sputum Collection Centre Plays a Significant Role in TB control Programme in Un-reached Tribal Areas of Orissa, India

Sashi Kanta Nayak, N.N. Sahu, P.U. Singh

Introduction: In 1997, the RNTCP programme was launched in Orissa, India and gradually covered all the districts of Orissa. It has now covered all 30 districts of Orissa with a population of about 36.8 million. Successful TB control programmes depend upon case detection, case holding and follow up. Poor socio-economic conditions, lack of awareness, remoteness of villages and lack of availability of services have negatively affected the control of the disease. Because a certain proportion of TB cases are left untreated, infection with the deadly disease continues in the area. For this purpose, Sputum Collection Centres (SCCs) have been set up in the remote and un-reached area of Koraput, the tribal heartland of Orissa, India.

Objective: The basic objective of the study is to find out the role and significance of SCC in TB control measures in tribal areas.

Methodology: 63 sputum collection centres were surveyed for this purpose with a questionnaire. General information on Sputum Collection Centres especially on distance, methods, transportation and follow-up have been compiled and compared with PHI data.

Salient Observations: 63 sputum collection centres (SCC) have been established in outlying areas that are some distance from the microscopic centres. Around 70% of the centres are situated more than 10kms from the microscopic centre and each centre caters for a population of about 4000 – 5000.

The table below represents symptomatics collected annually and the number of positives found in SCCs, indicating their contribution to TB control programmes.

Outcome:

- Increase in case detection and decrease in the time gap of the detection process and availability of the closest possible services.
- All categories of people, including the aged and women, avail themselves of the facilities.
- Increase in community participation and strengthening of inter-sectoral coordination.

Name of the District	No of SCC	No of villages covered	Population covered (approx)	Distance from the microscopic centres (%)		
				0-9 km	10-20km	>20 km.
Koraput	63	1587	350000	30%	44%	26%

YEARS	1998	1999	2000	2001	2002	2003	2004	2005	2006	Total
No of sputum collection centres functioning	25	35	40	45	50	60	62	62	62	62
No of chest symptomatics reported to SCCs	299	1040	1112	546	806	1161	1270	900	925	8059
Sputum positive cases diagnosed of SCCs	33	130	140	93	120	135	103	76	76	906

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T-SPOT.TB Offers No Advantage Over Tuberculin Skin Testing for Diagnosis of Tuberculosis in Young Children

Mark P. Nicol[1,2,3], Mary-Ann Davies[1,4], Kathy Wood[1], Mark Hatherill[1,2,3], Lesley Workman[2,3], Anthony Hawkridge[1,2,3], Monique Hanslo[2,3], Brian Eley[1], Katalin Wilkinson[2], Robert J Wilkinson[2,5], Willem A. Hanekom[1,2,3], David Beatty[1], Gregory Hussey[1,2,3]

Background: The enzyme-linked immunospot assay, T-SPOT.TB (Oxford Immunotech), is reported to be 83-93% sensitive for the diagnosis of active tuberculosis in children in hospital-based studies. We determined the performance of T-SPOT.TB in young children in a community setting.

Methods: Children from a rural community presenting to their local clinic or hospital with symptoms suggestive of tuberculosis or with a history of exposure to tuberculosis were admitted to a case verification ward. T-SPOT.TB was performed and children were investigated with clinical examination, tuberculin skin test, chest radiograph and culture of induced sputum and gastric lavage. The diagnosis was determined using a clinical algorithm.

Results: 243 children (median age 18 months) were recruited, of whom 214 had interpretable T-SPOT.TB results. The sensitivity of T-SPOT.TB was no better than tuberculin skin testing for the diagnosis of culture-confirmed tuberculosis (50% and 80% respectively) or clinically defined tuberculosis (40% and 52% respectively). The specificity of T-SPOT.TB was similar to that of tuberculin skin testing for the 88 children defined as unlikely to have tuberculosis (85% and 82% respectively). Children <12 months of age with a history of exposure were 11 times more likely than older children to have a negative T-SPOT.TB (p=0.0028), while TST was unaffected by age.

Conclusions: In young children presenting with symptoms suggestive of tuberculosis or being screened following exposure to tuberculosis in a community setting, T-SPOT.*TB* offered no advantage over tuberculin skin test for the detection of active tuberculosis. The test had very low sensitivity in children under the age of one year.

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In-house, Single-Tube Nested PCR for the Detection of TB in Children, Using Induced Sputum Samples

Felicity Leisegang, Heather Zar, Jabulani Ncayiyana, Mark Nicol and Howard Henderson

Background and Aims: We have recently shown that sputum induction is safe and useful for microbiological confirmation of tuberculosis in children, with improved yield over gastric lavage (1). We wished to determine whether rapid, cheap, in-house PCR, using sediment obtained from induced sputum specimens, could provide a rapid and accurate diagnosis of tuberculosis in children.

Methods: We performed an in-house PCR assay with sample blinding. The single-tube nested format was chosen, for the reasons of simplicity, sensitivity and diagnostic accuracy. The final assay is an adaptation of the methods of DNA extraction and PCR reported by other workers (2,3). The assay amplifies the IS6110 insertion element in the TB genome and was sensitive enough to detect 10 copies in each PCR tube. Frozen decontaminated induced sputum sediments from 240 children presenting to a tertiary hospital with suspected pulmonary tuberculosis were tested.

Results: Sputum samples from 240 patients [median (IQR) age 13 (6-24) months] were available for PCR. Of these, 54 (22.7%) were positive by culture or AFB smear, of which 26 (48%) were AFB positive. In two samples the presence of PCR inhibition was detected. In the remaining 238 samples, PCR was 83% sensitive for the detection of culture-confirmed TB, with a positive predictive value of 76%. Specificity was 92% with a negative predictive value of 95%.

	Culture +ve	Culture - ve	Total
PCR + ve	45	14	59
PCR - ve	9	170	179
	54	184	238

Three samples with positive PCR results had negative induced sputum results but a positive culture from bronchoalveolar lavage. If these were included in the analysis, the sensitivity of PCR was 84% and specificity 94%. PCR was 100% sensitive for smear positive culture confirmed TB and 71% sensitive for smear negative, culture-confirmed TB.

Conclusion: The reported performance of PCR for the diagnosis of tuberculosis varies widely. It is likely that assay and operator-dependent factors influence these reports. We have demonstrated that in childhood tuberculosis (which is typically paucibacillary and seldom smear positive) PCR is a useful addition to the diagnostic workup. If technical and feasibility issues can be overcome, PCR remains a promising tool for the diagnosis of tuberculosis in difficult clinical situations, such as childhood tuberculosis.

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Poverty vis-à-vis TB diagnostics. A Crisis for People Living with HIV/AIDS in Africa: Rethinking the Strategy for TB Control

Frederick Lawrence Okello

Background: Globally, and especially in Africa, TB is the most common infection and a leading cause of death among people living with HIV. Despite being curable, TB accounts for two million deaths each year. Among HIV positive people, 15% of deaths are TB-related1. Poor people are in the majority of the African population affected by TB; 95% of the disease is concentrated in the developing world2. Of the 14 million people co-infected with TB/HIV, 10 million reside in Africa. Scarcity of resources; neglect, ignorance and challenges posed by TB/HIV co-infection have collectively compounded a diagnostic emergency in TB, jeopardising people with HIV. HIV increases the risk of developing TB by 50% and this is the main agent for the global increase in TB prevalence, along with resistance to the most potent multi drug regimes (e.g. isoniazid and infampicin). Access to health care among the rural communities of sub-Saharan Africa poses the biggest threat to TB control. Most tuberculosis patients only have access to obsolete tests developed over a century ago due to lack of adequate government resources.

Objectives:

- To analyse the problems linked to the challenges posed by TB diagnostics in Africa.
- To underline other problems associated with TB surveillance, access to treatment and monitoring in Africa.
- To examine the possibility for consistency in detecting TB disease in HIV positive people.
- To examine ways of improving clinical examination and symptom assessment.

Method / Issue: The most frequently utilised TB diagnostics lack the sensitivity to consistently detect the bacteria in HIV co-infected population. The tests are also incapable of identifying drug-resistant strains of TB. TB diagnostics are often delayed in co-infected people and people with MDR TB, resulting in unnecessary illness and death. Rural communities in Africa have little or no access to general health care, let alone TB diagnostic and treatment centres with sufficient resources. A case study of Tanzania: in August, 2006, I conducted a study in Arusha and Manyara provinces of Tanzania for a local NGO Elderly Home Care, to establish the urban and rural communities' access to health services using both primary and secondary data.

Findings / Comments: In sub-Saharan Africa, there is a correlation between availability of diagnostic tests and access to the requisite resources needed to administrate tests, which include trained technical staff, clean water, electricity, funding for equipment, testing and maintenance. If testing is not free in Africa, very few patients may undergo these tests. The contemporary diagnostics recommended as a guide for health care providers in diagnosis of TB are inclined towards chest x-rays and smear microscopy, which do not address the particular challenges posed by HIV/TB co-infection. According to data obtained from secondary sources, about 25% of mainland Tanzania's households were 7km[3] or more from the nearest dispensary or health centre, while nearly 68% of the households are located 7km or more from the nearest hospital. Almost 90% of the households were within 10km of a dispensary or health centre. As would be expected, the distance to a hospital was greater. On average, 90% of rural households reported being 26km from a hospital.

The most common reason for not consulting a health provider was that there was no need, probably because the respondents often diagnosed and treated the condition themselves. Nearly 40% of households in urban areas and about 32% in the rural areas[4] respectively, reported that they did not consult a health provider because the services were too expensive. About 10% of households in rural areas did not consult a health provider because the facility was too far away. The older people were the most affected by the problem of access to health care (5% of the entire Tanzanian population is 65 or older).

Conclusion: The marked increase in HIV-associated TB globally, especially in Africa, demands rethinking the TB control strategy. A case in point is the directly observed, short course therapy (DOTS), introduced by WHO in 1993 and now used in 191 countries worldwide. DOTS resulted in a dramatic improvement over the period prior to 1993, when no global standardisation was articulated. Its marked success notwithstanding, DOTS is also limited - these tools focus on TB in the LUNG, and seek presence of bacilli in the sputum. DOTS is reliant on passive case finding, a handicap that is occasioned by patients with coughs lasting more than three weeks, who get a clinical exam and sputum smear microscopy. HIV-positive people have fewer bacilli in their sputum and are more likely to have extra-pulmonary TB. Consistent detection of TB in HIV-positive people therefore cannot be effectively accomplished by the most commonly used tests. Its is noteworthy that despite emerging technologies and innovations, a lot needs to be done to improve access

to health care in rural communities in Africa, which are among the most afflicted in the world. Access to health care is multi-faceted; it includes education, appropriate technology, equipment, personnel, infrastructure, resources and good governance. The main issue is thus to devise effective methods for improving TB control which demands, apart from other innovations, access to new and improved diagnostics and emerging technologies, which calls for a change of strategy in response to major challenges posed by TB and HIV co-infections in sub-Saharan Africa in particular and the world at large.

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1 Jayed Syed (www.thebody.com) 2 World Health Organisation (WHO) 3 The United Republic of Tanzania, 1991/92 Household Budget Survey Report 4 The United Republic of Tanzania, 2002 Population and Housing Census Report Institute of Data Sciences, Nairobi, Kenya

Gender Barriers to Tuberculosis Diagnosis

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Background: Tuberculosis (TB) kills more women than any other infectious disease. There is conflicting evidence concerning interactions between gender and TB. Great efforts have been made to improve TB diagnostics in resource-poor settings; however, even a perfectly rapid and sensitive diagnostic assay will not benefit those who do not present to the health services for testing. In many cultures, gender-specific issues prevent women from accessing health services and this may be a significant barrier to TB diagnosis and control.

Objectives: To characterise gender-related barriers to TB diagnosis in a Peruvian shanty town.

Methods: We studied published reports of gender associations with TB incidence and prospectively studied gender differences in TB health-seeking behaviour and diagnosis in 1 500 TB patients and their 3 500 household contacts over five years. In the light of these results, we performed an in-depth investigation of attitudes and experiences relating gender to TB diagnosis with 42 shanty town residents. The grounded theory approach was used in 21 semi-structured interviews with TB patients and health care workers. These issues were then further explored in focus group discussions with 21 TB patients and health care workers.

Results: Women were 0.7 times more likely to be diagnosed with TB than men both nationally ($P<0.0001$) and in the shanty town community that we studied prospectively ($P<0.01$). Consequently, 42% of TB was diagnosed in women. However, when we prospectively implemented active case detection in 3 500 household contacts of smear-positive TB patients and regularly visited and offered TB testing to those with symptoms, then there was no significant gender difference in TB diagnosis. Specifically, in active surveillance of TB contacts, 58% of those diagnosed with TB were women. When adjusted for sampling bias, the relative risk of TB in women was 0.9 such that 48% of TB was diagnosed in women. Thus, women were less likely than men to be diagnosed with TB within the national TB control programme, but this gender bias virtually disappeared within a population who were regularly interviewed in their homes and offered TB screening if they had cough.

Interviews and focus group discussions suggested that the TB programme was non-discriminatory, providing equal access and resources for men and women, and was also perceived by patients and potential future patients to be non-discriminatory. Men and women were similarly knowledgeable about TB and under what circumstances they would be recommended to seek diagnostic services and that these services were available free of charge. However, both genders almost universally expressed beliefs that female health was a lower priority than male health and that it was consequently less important that women seek TB diagnostic testing. This was attributed to the absence of a social safety net to provide income for unemployed families, combined with limited work opportunities for females within this cultural context.

Implications: There is a need to improve women's access to TB diagnostics and the greatest opportunity appears to be in social education more than programme modification, to tackle misconceptions about TB and to encourage women to present for investigation and diagnosis without the fear of ensuing stigma and abandonment. Furthermore, tackling poverty amongst women, increasing their employment and helping them to reduce financial dependence on men, may encourage gender equality in access to diagnostic healthcare services.

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Establishing TB Culture Facility to Tackle the Challenge of MDR and XDR TB in the Kingdom of Lesotho

C.N. Paramasivan

One of the most urgent needs for countries in sub-Saharan Africa, where TB is compounded by the HIV-AIDS pandemic, is to establish quality-assured, aerosol-free, safe TB laboratory networks for tuberculosis culture and drug susceptibility testing (DST). Among these countries, the Kingdom of Lesotho, with a population of 1.88 million, ranks among the worst affected, with an incidence of 236 infectious TB cases per 100 000 people.

As a rapid response to the emergence of MDR and XDR TB in the neighbouring South African province of KwaZulu-Natal, FIND conducted an assessment in November 2006 to evaluate the TB laboratory facility in Lesotho in consultation with the WHO. This evaluation revealed that the designated National Reference Laboratory (NRL) in Maseru had (a) had a shortage of qualified personnel; (b) a lack of technical know-how and training; (c) a shortage of equipment and financial resources; and (d) needed assistance to organise a quality-assured TB culture facility. The country-wide, organised quality-assurance programme (QAP) for sputum microscopy, as well as guidelines and standardised training for such activities, were also in need of technical and financial support. FIND and Partners in Health (PIH) initiated a two-year collaboration with the Ministry of Health and Social Welfare (MoH&SW) with the aim of establishing the NRL and to streamline culture and DST facilities in the country. FIND prepared detailed technical and resource requirements to create a functional NRL and PIH provided the necessary financial support, human and other resources. In addition, the FIND team in Geneva and a FIND consultant, who was posted at the NRL in May, 2007, coordinated and provided necessary supervision and technical expertise.

The NRL at Maseru was renovated between May to July, 2007 to meet all the basic laboratory requirements such as media preparation, sterilisation, a containment negative pressure facility for culture and drug susceptibility testing (DST), and also smear microscopy. The floor plan of the existing laboratory was re-modelled; and a culture facility was created for both solid and liquid culture systems. Engineering controls, such as a continuous negative air pressure system with a HEPA filtered air source to supply more than 10 air exchanges per hour, and an on-site sterilisation system, were established. All the necessary equipment procured under GFATM funds were made functional inside the facility.

Simultaneously, on-site evaluation visits of peripheral laboratories were carried out for external quality assessment in smear microscopy. Guidelines for the national laboratory network for quality assurance (QA) of smear microscopy, and training manuals and a module for laboratory technicians were developed. A rechecking programme is under way. The NRL, which also functions at present as a routine microscopy centre, initiated the rechecking programme from August, 2007. QA orientation trainings to all the laboratory technicians (LTs) in the country were scheduled to begin in September, 2007.

Standard operating procedures for culture and DST were introduced. Four additional LTs were recruited, and they were trained in August 2007. With the existing but limited resources, the lab has demonstrated adequate capacity and proficiency in carrying out cultures on solid egg-based LJ media. In a 20-day period in August, 175 sputum specimens, including 100 smear positives, were processed. All smear positive specimens were also set up for direct susceptibility testing for rifampicin. A standard recording and reporting system has been established. International proficiency testing for the culture and DST programme has been initiated. Twenty unknown cultures were obtained from MRC, Pretoria, for this purpose. After demonstrating expected level of proficiency in LJ media, MGIT liquid culture and DST was to begin in October, 2007.

In conclusion, the NRL at Lesotho has been established within a short period of time without compromising the internationally acceptable standards and demonstrated adequate proficiency in carrying out culture and DST on solid LJ media. This very rapid and cohesive response to a need for improved laboratory preparedness was made possible by the proactive collaboration of all concerned agencies, together with the government of Lesotho.

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Sensitivity of QuantiFERON-TB Gold In-Tube in Zambian Adults with Smear Positive Tuberculosis

Edward Raby, Akash Devendra, Helen Ayles, Peter Godfrey-Faussett

Background: Interferon gamma release assays (IGRA) are replacing the tuberculin skin test (TST) as a diagnostic tool for Mycobacterium tuberculosis infection. However, research into the test's performance in the high HIV-TB burden setting is scarce.

Aim: To define the sensitivity of an IGRA, QuantiFERON-TB Gold In-Tube (QGIT), in adult Zambian patients with active smear positive tuberculosis. Secondary outcomes focused on the effect of HIV on the test's performance.

Methods: Patients recruited were tested with QGIT and T lymphocyte estimations were performed ($CD3^+$, $CD4^+$, $CD8^+$).

Results: 47 subjects had a valid QGIT result. Overall sensitivity was 61.7% (95%CI: 47,76). A marked decrease in sensitivity was observed in HIV-positive compared to HIV-negative patients (42% v 80%, chi^2 p=0.019). The proportion of positive results decreased at CD4 counts <200 cells/μl (33% v 72%, chi^2 p=0.41) with an increase in both indeterminate and false negative results. Of six subjects with $CD4^+$ counts <100 cells/μl none had a positive result.

Conclusions: Where HIV is prevalent, an increased proportion of indeterminate and false negative QGIT results should be expected in patients with active TB. The implications of this for the diagnosis of LTBI by QGIT are unclear. The diagnostic and prognostic relevance of IGRAs in high burden settings needs to be better characterised.

London School of Hygiene & Tropical Medicine

The South African Demonstration Project on the Use of a Rapid MDR-TB Assay for Routine Diagnosis of MDR-TB under TB Control Programme Settings

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Background: The spreading epidemic of MDR-TB threatens global TB control. The more recently described problem of XDR-TB, especially when linked to HIV infection, is of even greater concern. These problems are especially severe in South Africa that is home to 20% of the world's HIV infected TB patients and has the largest burden of reported MDR-TB in the world. The conventional diagnosis of drug-resistant TB is based on culture and drug susceptibility testing (DST) on liquid or solid media with results available in weeks to months. During this time, transmission of disease continues and many patients die. Furthermore, the long delay in the initiation of appropriate treatment leads to clinical deterioration and eventually to poor treatment outcomes.

A number of novel assays for rapid diagnosis of drug resistance have been developed, of which some are commercially available and routinely used in TB low prevalence settings. However, good performance under controlled laboratory investigations does not automatically lead to effectiveness under routine diagnostic conditions. High quality laboratory, operational, cost, and clinical assessment of the assays in populations and conditions of intended use are necessary to ensure that performance characteristics are well understood. The greatest need for these assays is in low-medium income countries, with high TB burden, and where HIV prevalence is also high. The effectiveness of rapid assays for diagnosis of MDR-TB is determined through demonstration projects. These projects are designed to investigate the performance of the new diagnostics under large scale implementations in programme settings and also aim to provide evidence for policy, and to ensure for the rapid adoption of useful technologies in Control Programme practices. The SA MRC, NHLS and FIND are collaborating on a large scale demonstration project for rapid MDR TB diagnosis. We describe the project and the lessons learned to date in project implementation.

Objectives: The objectives of the project are to assess the feasibility and impact of a rapid test for detecting MDR-TB in individuals at risk of MDR-TB in a high MDR-TB-HIV burden setting. The feasibility of implementation, cost, cost-effectiveness, and clinical impact of the assay under NTP conditions will be determined. Furthermore, should the demonstration project prove effectiveness, we aim to provide evidence to the NTP on how the test may be best applied in diagnostic algorithms and confirm the benefits (including financial) from implementation.

Methods: The project is conducted as a prospective observational cohort study, whereby sputum specimens are collected from TB suspects and patients at high risk for MDR-TB. Enrolment is taking place in four provinces, with rapid assays being performed at the national and provincial NHLS laboratories. The enrolment target is 10 000 specimens over a one-year period. Both conventional culture and first-line DST and the rapid assay are performed on each specimen. Patients will be managed according to the result of the rapid assay and final management will be based on the conventional result. Patient safety is ensured through certain procedures. Data analysis will focus on assay performance, impact on laboratory, patient management and on health facility procedures, clinical indicators, and cost-effectiveness and cost indicators and on the appropriate place of rapid assay in case finding algorithms. The framework for adoption, introduction and implementation of new technologies for TB is followed to ensure roll-out of the new assay into NTP policy and practice after completion of the demonstration project.

Findings: Challenges and lessons learnt during the implementation phase centred on conducting a large national scale research project according to strict time lines, data management, bias in sample selection and ethical issues. Integrating different levels/partners such as the NTP, provinces, health facilities and laboratories into a large scale research project, and ensuring the involvement of key stakeholders had also to be achieved. Stakeholder perception, expectations and satisfaction also need to be managed. Provincial differences in capacity for implementation were assessed.

Conclusion: The demonstration project is expected to provide detailed information on the feasibility and requirements for implementation of rapid assays for routine diagnosis of MDR-TB, impact on patient management, and cost and cost-effectiveness in early 2008. The demonstration project is an important step in the process of ensuring that new technologies are adequately evaluated

in the field and that recommendations to NTPs on their rational use can be made. Key stakeholders are involved to allow for roll-out of the new assay into NTP policy and practice after evaluation to ensure that the country continues to receive benefit from rapid diagnosis of MDR-TB. Furthermore, the data will also be presented to the WHO Strategic and Technical Review group for consideration for adoption as WHO policy for use of such assays in other high burden settings.

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Treating Intestinal Helminths Augments Anti-mycobacterial Immunity, Converting Interferon-gamma Release Assay Diagnostic Tests for Tuberculosis Infection from Negative to Positive

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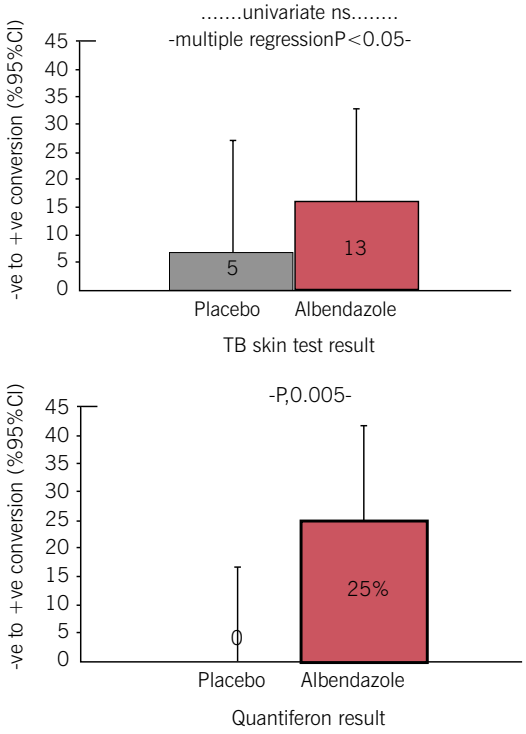
Background: Interferon-gamma release assays are increasingly used for diagnosing tuberculosis infection and disease and the QuantiFERON-gold in-the-tube assay is suitable for use in field settings. This assay has been evaluated principally in tuberculosis non-endemic countries. Intestinal helminth infections have similar geographical distribution to tuberculosis and helminths bias the immune system away from the cell-mediated immunity that is required to control intracellular infections. We therefore hypothesised that treating intestinal helminths would augment anti-mycobacterial immunity and modulate interferon-gamma release assay results.

Methods: A double-blind, randomised, placebo-controlled trial was performed in 144 healthy adults in the Peruvian Amazon. Anti-mycobacterial immunity was quantified *in vivo* using tuberculin skin-testing and *in vitro* by quantifying interferon-gamma secretion in response to whole-blood stimulation with tuberculosis antigens. These tests and stool parasitology were performed at recruitment and four weeks after placebo or deworming with three daily doses of 400 mg albendazole.



Results: Stool microscopy at recruitment for 126 participants diagnosed intestinal helminths in 48%. Forty percent were infected with *Ascaris lumbricoides*, 12% *Trichuris trichuria*, 6.3% hookworms, and 3.2% *Strongyloides stercoralis*. Tuberculin skin test responses increased in size following albendazole therapy (P=0.03) but not after placebo, demonstrating that deworming augmented the immune response to tuberculin *in vivo.* By all criteria of tuberculin skin test conversion, deworming caused more conversions than placebo (Figure, left) and this was statistically significant in multiple regression (P=0.03) but not univariate analysis (P=0.1). Similarly, the *in vitro* quantification of anti-mycobacterial interferon-gamma responses to specific tuberculosis antigens (Figure, right) increased after albendazole therapy (P=0.02) but not placebo. Consequently, 38% (53/138) of baseline QuantiFERON assays were positive at recruitment, and albendazole caused 17% (9/53) of the initially negative tests to become positive, vs. 0/49 after placebo (P=0.003). The QuantiFERON in-the-tube assay proved to be practicable in this per-urban jungle shanty town setting for sample collection and initial processing, although the refrigerated product of the assay required subsequent ELISA analysis in an appropriately equipped laboratory.

Conclusion: Treating intestinal helminths augmented anti-mycobacterial immunity *in vitro* and *in vivo.* Thus antihelminthic therapy should be evaluated as a strategy for reducing tuberculosis susceptibility. Furthermore, helminth infections confound the interpretation of this interferon-gamma release assay for tuberculosis infection by leading to false-negative results, and deworming can reverse this phenomenon.

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Pheelo, healthcare worker from Lesotho, talks about the challenges of TB and HIV

TB DYING FOR A TEST

Session 3

Future Field Diagnostics

Immunogenicity Testing of New Potential Diagnostic Antigens of Mycobacterium Tuberculosis Infection by Whole Blood IFN-γ Release Assay in Three Distinct African Populations

Gillian F. Black, Bonnie Thiel, Gerhard Walzl, Henry Boom, Philip C Hill, Harriet Mayanja, Kim Stanley, Keith Chervenak, Kees L.M.C. Franken, Annemiek H. Friggen, Michel R. Klein, Martin Ota, Tom H.M. Ottenhoff, Shreemanta K. Parida, Stefan H. E. Kaufmann and the GC#6-74 Consortium

GC#6~74 Consortium on "Biomarkers of Protective Immunity against Tuberculosis in the context of HIV/AIDS in Africa" is supported by The Bill and Melinda Gates Foundation. The consortium consists of 15 partners across Europe, the US and Africa, including seven field sites in five African countries with endemic tuberculosis (TB). This ambitious project is focused to elucidate differences in the immune response between people exposed to TB who never become sick and those who develop TB both within and without the context of HIV/AIDS. The aim is to define biomarkers of protection and disease for diagnostics, clinical testing of novel drugs and vaccines for TB. Within the first two years of the project we have effectively established a functional, cohesive, multi-centered and multi-cultural team. We are progressing well with recruiting longitudinal cohorts of HIV-negative and positive latently infected TB contacts, as well as TB index cases. These cohorts are being followed up at multiple time points for immunological and clinical evaluations for a total of two years.

The sequencing of the *M. tuberculosis* (MTB) genome and gene expression studies have led to the identification of proteins that could serve as novel diagnostic agents for infection with *M. tuberculosis*. Screening of 86 proteins, including novel antigens associated with latent TB infection and reactivation TB, as well as all the major known TB antigens from different published studies, has been completed at three of the African field sites (South Africa, Uganda and The Gambia). These antigens have also been screened at four other field sites and a final consensus on the selection of the first set of antigens to be included in the longitudinal cohort studies has been reached. Here we present IFN- responses measured by ELISA in supernatants of 7-day whole blood cultures stimulated with up to 57 novel or known MTB proteins plus controls. A total of 139 tuberculin skin test positive (>10mm induration), HIV- negative adult household contacts (HHC) of active pulmonary TB cases from South Africa (SA; n=60), Uganda (n=53), and The Gambia (n=26) were tested with up to six classical TB specific antigens (ESAT6, Ag85, HSP65, TB10.4, Tesat/CFP10, Rv3019c (TB10.3)) and 51 so-called latency (DosR regulon) recombinant proteins which were all cultured at a final concentration of 10ug/ml. All antigens were produced, quality controlled and distributed by Leiden University Medical Center and each African field site obtained the same batches. Immunological assays and clinical procedures have been harmonised to the highest possible level across the field sites.

Statistics were calculated on original pg/ml data, adjusted for the negative control, averaged over duplicate ELISA results and replicate assays (multiple assays on a single subject). Site specific response cut-offs were defined as the mean of the negative control values plus two standard deviations of the mean. Responses to the negative control (culture medium RPMI only) were low in South Africa, The Gambia and Uganda, with a combined mean of 10pg/ml.

In study participants from all three countries there were high frequencies of response against the positive control PHA, with 72%, 96%, and 59% positive responses in SA, The Gambia and Uganda. The ESAT6/CFP10 fusion protein was the most frequently recognised antigen evoking responses in >70% of study participants in all three countries. ESAT6 was tested in SA only and 70% of study participants responded. Antigen 85 was tested in SA and Uganda. Preparations of the recombinant antigens Ag85A and Ag85B were combined in equal amounts prior to culture to form a single stimulatory condition; the final concentration of protein was 10ug/ml. In South Africa, only 7% of study participants produced a response to Ag85A/B, whereas in Uganda this combined antigen was recognised by a much higher proportion of individuals (56%), although the mean magnitude of response was not strong. The least frequently recognised control antigen tested across all three field sites was HSP65, followed by TB10.3 and TB10.4. Of the 51 latency antigens that were tested, the same antigen was most commonly recognised by participants from SA and Uganda. The same antigen was the 2nd most commonly recognised antigen in The Gambia. The mean magnitude of response to this antigen varied between the three sites, being strongest in Uganda and lower in The Gambia and SA. The top three most commonly recognised antigens in South Africa were among the top five most commonly recognised antigens in Uganda.

The most commonly recognised antigen in the Gambia was recognised by no SA contacts, and by one out of 29 people tested in Uganda. There was good agreement between Uganda and SA with respect to the antigens provoking the highest percent of responders and the highest magnitude of response with some agreement between the data of both these countries and The Gambia.

Appreciable percentages of TST positive TB contacts with very high likelihood of latent infection with *M. tuberculosis* responded by IFN-γ production to five or six novel DosR regulon MTB proteins. Although there are encouraging similarities in antigen recognition between the three sites, there are also important differences that may stem from the fact that the three population groups included are distinct. The initial results observed from these studies are promising and some of the antigens tested may have the potential for inclusion in the next generation of specific immunodiagnostics tools for TB. Our current ongoing longitudinal cohort studies will throw more light on these issues and we expect to obtain the first set of results from the first cohorts by early 2008.

GC#6-74 Consortium on "Biomarkers of Protective Immunity against Tuberculosis in the context of HIV/AIDS in Africa"

The Mulago Inpatient Noninvasive Diagnosis of Pneumonia Study: A Platform for Investigating Novel TB Diagnostics

J. Lucian Davis[1], William Worodria[2], Adithya Cattamanchi[1], Samuel Yoo[3], Richard Musinguzi3, Alfred Andama[4], John Kiidha[3], Francis Adatu[5], Phillip Hopewell[1], Laurence Huang[1]

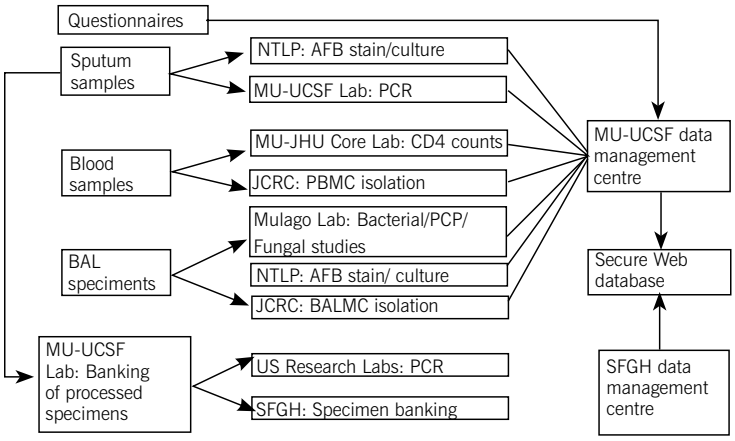
Background: Recently, the substantial increase in funding for TB diagnostics has led to an increasing number of published studies. However, the overall quality and methodological rigour of these studies have been questioned, raising concerns that published data will not predict the bedside performance of new TB diagnostics. Common methodological problems include small sample sizes, non-consecutive or non-random patient enrollment, lack of an adequate reference standard, and lack of prospective clinical follow-up. With many TB diagnostics now in advanced phases of development, establishing representative clinical cohorts and specimen banks linked to rigorous clinical data is essential to evaluating their performance. Such data will facilitate bringing assays to market quickly, and ultimately will improve the care of TB patients.

Objectives: To describe the infrastructure and preliminary epidemiologic results from a prospective clinical cohort designed to evaluate novel diagnostics for TB and HIV-associated pulmonary diseases at Mulago Hospital in Kampala, Uganda.

Methods: All patients clinically suspected of pneumonia and symptoms > 3 weeks are screened and enrolled on admission from the emergency room to the medical ward of Mulago Hospital. Demographic and baseline clinical information are collected and chest radiographs are digitised. Initial laboratory evaluation for all patients includes HIV testing; CD4 count measurement; and AFB smear (direct Ziehl-Neelsen and concentrated auramine-O) and culture of two sputum specimens (collected on hospital days one and two) performed at the Uganda National Tuberculosis and Leprosy Programme (NTLP) reference laboratory. On hospital day three (or as soon as possible), HIV-infected patients with negative sputum AFB smears undergo bronchoscopy with broncho-alveolar lavage (BAL). BAL specimens are evaluated with Gram stain and culture (?); AFB smear and culture; Giemsa stain for Pneumocystis; and fungal stain (potassium hydroxide) and culture. After hospital discharge, all patients are evaluated at two months to adjudicate final diagnoses based on clinical status and response to therapy. Blood and sputum specimens are collected at the follow-up visit. Samples of all blood (serum and peripheral blood mono-nuclear cells), sputum, and BAL (supernatant and BAL mononuclear cells) specimens are banked for future studies.

Results: Infrastructure to manage clinical data, analyse laboratory specimens, and bank specimens is detailed in the figure below. Between April and June 2007, 209 patients met study eligibility criteria, 86 of whom were enrolled (random sampling in initial two months; consecutive sampling thereafter). 86% were HIV-positive with a median CD4 T-cell count of 54 cells/mm3 (IQR 14-156). 61% had been previously diagnosed with HIV. Of these, 87% were taking co-trimoxazole prophylaxis and 20% were taking anti-retroviral therapy (ART). The majority (70%) had not received previous antibiotic treatment. After final evaluation, 36 (42%) patients were shown to have microbiologically confirmed TB, of whom 2 (2.3%) required bronchoscopy to establish the diagnosis. Follow-up evaluation was completed in 61% of those alive at discharge.

Conclusions: We have successfully developed an infrastructure to evaluate novel diagnostics for TB in a resource-poor setting. The ongoing study has several strengths, including consecutive sampling of a relevant population, those with pneumonia of unknown etiology; thorough diagnostic evaluation of all patients, including bronchoscopy when appropriate; prospective clinical follow-up to adjudicate outcomes in patients without microbiologically confirmed disease; and banking of clinical specimens for future studies. Preliminary data shows a high prevalence of TB and HIV, making the MIND study an ideal platform for evaluation of a variety of TB diagnostics including antigen-, antibody-, PCR-, and immune-based assays.



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Low-cost Incubator Designs for Tuberculosis Culture Diagnosis in Resource-poor Areas

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Background: Tuberculosis is an aerobic slow-growing bacterium with high prevalence in resource-poor regions. Laboratory drug-susceptibility testing and also sensitive diagnosis for sputum smear-microscopy negative patients both usually require a 37ºC incubator for culture. The problem with these incubators is that they are very expensive and are difficult to purchase, often needing international importation to the economically disadvantaged areas where most TB occurs and where they are most needed.

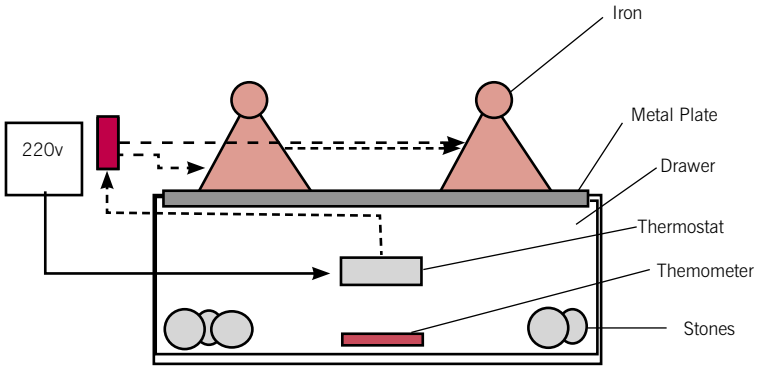
Objective: To develop designs for low-cost, reliable incubators 'home-made' from non-specific commercial materials that could bring alternative choices to high endemic areas where a commercial incubator cannot be bought or properly maintained and where specific needs such as intermittent electricity supply must be considered.

Methods: A prototype (Figure) uses two irons, a thin metal plate and an electrical thermostat removed from a household fan heater. This all functions at 'mains' electricity voltage (220V or 110V) and can be fitted in a regular drawer, cupboard or large box. The irons, adjusted to a low-warm setting, were put on top of the metal plate in order to distribute the heat evenly throughout the incubator. Some stones were put inside to act as a buffer to reduce temperature variation, in place of the water jacket that is often used in commercial incubators. The thermostat was put into the centre of the incubator, so it can act as a switch to turn on the irons or shut them down if necessary. To check the temperature we used a standard mercury thermometer. An electric heating blanket designed for use on a bed also can be used to heat the incubator and has the advantage of always being adjustable to low body temperature, which some irons are not. However, electric blankets are not as widely available in tropical countries as electrical irons, so the designs are complementary, adaptable to different countries depending upon locally available materials.

Discussion: A 37ºC homemade incubator can be made from materials found in common stores. It has acceptable precision in maintaining 37ºC and has the advantage of being adaptable to different areas, according to space availability. Temperature control is not only done by the iron or electric blanket thermostat but this is backed-up by the thermostat removed from the fan heater to give extra security to ensure that the incubator does not overheat. Biosafety is an important issue but all the cultures are double-sealed: they only require a space at body temperature for culture. Carbon dioxide sources can be used in a low-cost incubator in the same way as in commercial incubators, but these only slightly improve tuberculosis culture, necessitate the biohazard of having open tuberculosis cultures and are rarely used in the resource-poor settings where most tuberculosis occurs. In hot countries, the incubators must be kept in a cool place so that the temperature does not exceed 37ºC on hot days, but this is true of all incubators.

Current Work: An alternative prototype under development uses a 12V heater removed from a scrap car and two car batteries, a transformer to use mains power when it is available and a $10 solar panel, marketed to rest on the dash-board of cars to top-up their batteries when parked. This design has the disadvantage that it uses parts that are not so readily available internationally, but it has the advantage of keeping the cultures at the right temperature even when the mains power is intermittent, for example in the jungle. The batteries keep the temperature constant through the night and are charged when the sun is shining or the mains power is available.

Implications. These mains-powered and solar-powered incubators are not products for sale but rather open-access designs that we wish to publish so that any basic laboratory can use minimal funds to adapt readily available items to convert a cupboard into an incubator so they can offer tuberculosis culture to their patients.



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Molecular Beacons: Rapid Detection of Mycobacterium Tuberculosis and Drug Resistance in Specimens from Developing Countries

Edward Desmond[1], Grace Lin[1] and Nancy Harris[2]

Background: Inadequate resources and expertise in mycobacteriology laboratories of some developing countries may hamper early detection of drug-resistant tuberculosis (DR-TB). Molecular beacons with their high specificity and sensitivity offer rapid detection of drug resistance mutations in real-time polymerase chain reaction (PCR) assays.

Objectives: To evaluate the performance of molecular beacon probes in detecting *M. tuberculosis* and mutations associated with resistance to isoniazid and rifampin. Specimens were sputum samples from U.S.-affiliated Pacific islands and the Tibet Autonomous Region (TAR) of the People's Republic of China.

Methods: Molecular beacon testing was performed as previously described (Lin, et el. 2004. J. Clin. Microbiol. 42(9):4204-8), using acid-fast smear positive specimens. Eight acid-fast smear positive specimens from the TAR, from a set of 25 sputum samples, were tested by molecular beacons, with follow-up culture and drug susceptibility testing using the Mycobacterial Growth Indicator Tube (MGIT). In addition, 50 smear positive specimens from Republic of Marshall Islands, and 42 from Federated States of Micronesia were tested by both molecular beacons and drug susceptibility testing. Molecular beacon testing was performed twice per week.

Findings: Pacific island specimens: 92 specimens were acid-fast smear and culture positive. Molecular beacons testing was performed on the smear positive specimen sediment within a few days of receiving the specimen. Five specimens that were smear negative were culture positive for *Mycobacterium tuberculosis* but were not tested by molecular beacons. 27 specimens were acid-fast smear positive and positive for the presence of *M. tuberculosis* complex by molecular beacons, but the culture was either negative or overgrown by nonmycobacterial contaminants. For the 92 specimens that had positive smears, cultures and molecular beacons testing indicating *M. tuberculosis,* two were resistant to INH alone, two were resistant to rifampin only, and four were resistant to both INH and rifampin. There was 100% agreement between molecular beacons results for INH and rifampin from specimen sediments and MGIT drug susceptibility testing performed using pure isolates of *M. tuberculosis*.

TAR Specimens: All eight of the acid-fast smear positive cultures were culture positive for *Mycobacterium tuberculosis.* In seven of the eight, molecular beacons detected the presence of *M. tuberculosis* and provided preliminary drug susceptibility testing results. One specimen that had only three organisms per 100 microscopic fields was negative on molecular beacons testing. Molecular beacons detected the presence of mutations associated with rifampin resistance in five specimens. MGIT drug susceptibility testing of culture isolates confirmed resistance to rifampin (and multidrug-resistance) in four of the five specimens. DNA sequencing of the rpoB gene for the fifth strain showed the presence of a mutation in the rpoB gene which is not associated with rifampin resistance. Four out of seven specimens were multidrug-resistant (resistant to at least isoniazid and rifampin).

For both sets of specimens, median turnaround time for molecular beacons results was four days. Median turnaround time for culture-based drug susceptibility testing was 47 days.

Conclusions: Molecular beacons testing provided rapid, accurate identification and drug susceptibility results for acid-fast smear positive specimens. Overall, in four years of experience comparing molecular beacons results with drug susceptibility testing by culture for INH and rifampin, agreement between the two methods has been 95.6% for INH and 96.7% for rifampin. Using this technique, patients with MDRTB can be quickly identified and started on an appropriate treatment regimen, reducing their period of infectivity. Quick detection of MDRTB also allows second-line drug susceptibility testing to be initiated promptly, so that extensively drug-resistant tuberculosis (XDRTB) can be more quickly identified. One patient from TAR had XDRTB, resistant to capreomycin, amikacin and levofloxacin as well as INH and rifampin. If new molecular beacons are developed, the method offers the promise of being able to detect resistance to aminoglycosides and fluoroquinolones as well, enabling detection of XDRTB directly in clinical specimens. The 27 Pacific island specimens which were positive by acid-fast smear and molecular beacons, but did not yield a culture positive for *M. tuberculosis* may represent a problem with stability of the bacteria when transported over long distances. Molecular beacons may be useful in this circumstance for detecting nonviable bacilli in specimens from new, untreated patients.

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Enhanced Ex Vivo Stimulation of Mycobacterium Tuberculosis-Specific T Cells in HIV-infected Persons via Antigen Delivery by the Bordetella Pertussis Adenylate Cyclase Vector

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Background: *Mycobacterium tuberculosis* is a significant threat to global health. The *M. bovis* BCG vaccine provides only partial protection and the tuberculin skin test (TST), used to aid diagnosis, lacks both specificity and sensitivity. The genetically detoxified *Bordetella pertussis* adenylate cyclase is a promising delivery system for immunodominant tuberculosis antigens in interferon gamma release assays. However, this system has not been evaluated in HIV-infected persons in high tuberculosis prevalence areas, or in tuberculosis endemic countries.

Methods: A whole blood interferon gamma release assay using *mycobacterium tuberculosis* antigens (early-secreted antigenic target-6, culture filtrate protein-10, alpha-crystallin 2, and TB10.3) delivered by adenylate cyclase in addition to native tuberculosis antigens (without adenylate cyclase delivery) was evaluated in 119 adults in Khayelitsha township, Cape Town, South Africa. Results were compared to tuberculin skin test in 41 HIV-positive and 42 HIV-negative asymptomatic persons in addition to 36 HIV-positive persons with recently diagnosed smear/culture positive pulmonary tuberculosis.

Results: Delivery of tuberculosis antigens by adenylate cyclase decreased by 10-fold the amount of antigen required to restimulate T cells. Furthermore, the responses of HIV-positive persons with a low response to native tuberculosis antigens were enhanced when these antigens were delivered by adenylate cyclase. When interferon gamma responses to the tuberculosis antigens (with or without delivery by adenylate cyclase) were combined, a significantly higher number of patients were scored positive compared to tuberculin skin testing.

Conclusions: *Ex vivo* responses to tuberculosis antigens delivered by adenylate cyclase are maintained in the context of HIV infection. Our findings suggest that the majority of this population are infected with tuberculosis, which is of significant public health importance.

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Lam-ICT – Point of Care Test for Mycobacterial Infections

Molokova E., Moody L., Grant J., Koulchin V.

Introduction: Tuberculosis (TB) is a multifaceted disease and a challenging public health problem in both industrialised and developing countries. Early diagnosis is essential to prevent the further spread of TB, but lack of screening diagnostic tools makes this task very difficult. The situation is exacerbated by a broad onset of TB/HIV co-infection epidemic in countries with high TB prevalence.

Earlier, Chemogen developed a sandwich immunoassay detecting LAM antigen in biological samples in a 96-well plate ELISA format. The 96-well plate LAM-ELISA is an immunoassay adapted for a moderately complex laboratory environment. In an attempt to provide a diagnostic tool for the point of care (POC) environment, Chemogen has developed a LAM-specific sandwich immunoassay in the lateral flow membrane format – LAM-ICT (dip-stick like test).

Principle of the Test: The rapid direct antigen test for TB in ICT format is specific to the major polysaccharide antigen of genus *Mycobacteria* – lipoarabinomannan (LAM).The test utilises highly purified rabbit polyclonal antibodies isolated by LAM-specific affinity chromatography. The same antibodies are used as the capture Ab and the detection Ab. The capture antibodies are adsorbed onto the nitrocellulose membrane of the ICT strip. The detection antibody is labelled by conjugation to colloidal gold particles and such conjugate, in a protective drying buffer, is dried down on the combined conjugate/sample pad. Antibodies adsorbed onto the ICT strip capture the LAM antigen present in the test samples. The conjugated antibodies then attach to the captured antigen, creating an immobilised immunological complex visible due to the presence of the colloidal gold label. A positive result (a visible red line) indicates that LAM antigen of *Mycobacteria* is present in the sample; whereas a negative result (no visible red line) indicates that it is not present at the test's detection limits.

The LAM-ICT assay, like LAM-ELISA, is designed to be used with urine samples from TB-suspected patients. Samples are heated at 100°C for 30 min and then centrifuged at 6 000 – 10 000 rpm for 10 minutes. Clear supernatant (150 microliter) is then applied to the ICT strip. The LAM-ICT has sensitivity in the range of 0.2-0.3 ng/ml of purified LAM antigen or approximately $5x10^3$-10^4 cfu/ml of the whole cells of *M.tuberculosis* spiked in urine. This is equal to the sensitivity of LAM-ELISA.

The LAM-ICT assay, like LAM-ELISA, does not differentiate between species of Mycobacterium, such as *M.tuberculosis, M.leprae,* and *M.avium.* In most cases, the presence of the LAM carbohydrate surface antigen in a clinical specimen indicates a mycobacterium infection due to *M. tuberculosis,* which must be followed up with a confirmation test such as bacterial culture. Because the LAM-ICT test detects the LAM carbohydrate surface antigen, the results are not limited by the status of the patient's immune system.

Clinical Performance: Results of clinical evaluation of the LAM-ELISA test show that the test is most efficient in the epidemiological setting with high prevalence of HIV/TB co-infection. In studies conducted in Tanzania in 2003 and 2005, sensitivity of the LAM-ELISA was equal to 80 – 84% for HIV/TB co-infected patients. Analysis of the data obtained in the Tanzania 2005 study demonstrates that TB and HIV co-infected patients with lower CD4 count have, in general, a significantly higher concentration of LAM antigen in urine. Concentrations of urinary LAM ranged from 0.1 ng/ml to several hundreds of ng/ml, with concentrations in the range of 0.1-0.3 ng/ml corresponding up to 10-20% of the study group. Specificity of LAM-ELISA was equal to 96-99% among TB-suspected, but confirmed to be TB negative patients. It was shown that concentrating urine samples using microcentrifugal ultrafiltration devices significantly increases test sensitivity without impacting its specificity.

A retrospective comparison of the LAM-ELISA and LAM-ICT using stored, frozen, non-concentrated urine samples from TB patients showed excellent correlation between the two assays starting from moderately low positive to higher positive samples (LAM-ELISA signal with OD_{450}>0.4). We suggest that use of the concentrated urine samples with LAM-ICT devices will significantly improve test sensitivity up to, potentially, a level of 85-95% in TB/HIV co-infected patients.

We believe that LAM-ICT can be successfully used in the POC environment in countries with a high level of TB/HIV co-infection.

Chemogen, Inc., So. Portland, ME, USA

Pilot Study on the Efficacy of Beta Galactosidase Reporter Phage for Rapid Field Diagnosis of Tuberculosis from Sputum Samples

Gomathi, N.S., Jordan Kriakov, Vanaja Kumar

Background: Effective control of tuberculosis depends largely upon early diagnosis of smear positive cases and their drug susceptibility detection. Among the many rapid diagnostic assays developed in recent years, phage based reporter assay has emerged as a promising one. Here, we report the preliminary findings of a pilot study conducted using beta-galactosidase reporter phage constructed from a temperature sensitive mutant of mycobacteriophage TM4. The advantage of a temperature sensitive mutant is that, at its restrictive temperature, it behaves like a temperate phage, where the cell is protected from lysis due to incorporation of the phage genome into the host genome. This results in extended expression of the reporter gene resulting in increased sensitivity of detection of the viable cells using the gene product.

Principle: Upon infection by beta-galactosidase reporter phage viable M. tuberculosis cells express the enzyme, resulting in the development of blue colour in the presence of the substrate X-gal.

Method: The present study included 43 smear positive and seven smear negative samples, processed by modified Petroff's method. Broth cultures were set up for each sample in modified Kirchner's medium without phenol red and incubated at 37oC. The assay was done with the cultures on day seven. Phage was added to cultures, followed by the substrate Xgal at 40 μg/ml concentration and incubated overnight. Three controls, namely, the assay control consisting of the phage and Xgal, positive control consisting of M. smegmatis mc2155 cells, phage and Xgal and negative control consisting of medium and Xgal were included. Any colour developed on the next day was visually compared with the assay control.

Results: All the 43 smear positive samples developed blue colour with 36 of them showing deep blue, six medium blue and one light blue. Among the seven smear negative samples, two produced deeper colour in comparison with the assay control. Thus the sensitivity of the assay was found to be 100% where as the specificity was found to be 71% only. This discrepancy could be explained by the limitation of the conventional smear, which requires 10^4 organisms per ml of sputum for detection. However, the specific nature of the mycobacteriophages enables them to produce visually detectable blue colour with $<10^4$ organisms per ml of sputum.

Conclusion: The assay has the potential to be used as a simple screening test in the field where mere addition of sputum to lyophilised phage and X-gal should indicate positivity through development of colour. Further study with larger number of samples, comparing the results with conventional culture and quantifying the colour developed could aid in field application of the assay.

Tuberculosis Research Center, Chennai

Optimised LRP Assay for TB Diagnosis

Vanaja Kumar

Background: The lack of accurate, robust, and rapid diagnostics impedes tuberculosis patient management and disease control. More than one type of test is needed for the different levels of the health care system as well as for different levels/categories of disease. A point-of-care test is required for use at primary health care level where the majority of patients seek medical attention but where diagnosis is currently based on clinical signs and symptoms only. At the peripheral laboratory level or district hospital, an alternative to microscopy, with a simpler technology that can detect both the smear-positive and smear-negative tuberculosis is required. At the district and national reference laboratory level there is a need for faster substitutes to culture for detecting smear-negative tuberculosis, improved antibiotic susceptibility testing, and tests for the detection of latent infection. Luciferase reporter phage assay has the potential for developing both field-friendly screening test as well as highly sensitive assay suitable for a sophisticated laboratory.

Luciferase reporter mycobacteriophages genetically engineered to carry firefly luciferase gene are highly specific for *Mycobacterium tuberculosis*. But the sensitivity of the assay is poor, picking up only 46% of positives as compared to conventional method of growing tubercle bacilli on Lowenstein Jensen slopes. Sputum specimens of tuberculosis patients contain normal and opportunistic flora colonising their respiratory system that can be controlled to some extent by use of antibiotics in the primary isolation medium. In addition, the presence of enzymes, inhibitory factors, remnants of mucous strands, epithelial and pus cells in the processed sputum deposits in varying levels hamper phage based assays in varying degrees, resulting in poor sensitivity. An attempt was made to improve the sensitivity of the assay by optimisation of the assay format.

Method: Optimisation of the assay format was done to improve detection by,

1. reconstitution of luciferin in DMSO that allows its transport into the host cell as such, in addition to its entry as protonated form in the acidic buffered solution;
2. dilution of sputum samples in Middlebrook 7H9 medium so as to adjust the proportion of phage to the unknown number of tubercle bacilli (MOI) present in sputum specimens and
3. addition of LRP a second time prior to the host cell lysis by the first phage infection.

The optimised LRP assay was done on sputum specimens processed by modified Petroff's method. One in 10 dilutions of sputum deposit (neat, N1 and N2) were prepared and transferred to 7H9 broth supplemented with polymixin, amphotericin, nalidixic acid, trimethoprim and Azlozilin (PANTA) and incubated. On days three, six and nine, phage was added to cells growing in the broth and the second addition of phage was done after 60 minutes. Photons liberated were counted as RLU after three hours, 24 hrs and 72 hrs incubation after second addition of the phage.

Results: In 76 sputum specimens from as many pulmonary tuberculosis patients, 29 specimens out of 34 culture positives (85%) were detected as positive by LRP assay. Out of 42 culture-negative samples 34 were categorised as negatives (81%). LRP assay picked up eight more positives compared to conventional culture on LJ, while it failed to detect five high-grade culture positives.

Conclusion: Modification of the procedure to include N3 dilution should lead to ideal multiplicity of infection (MOI) with high-grade positive specimens leading to better sensitivity of the assay. There is plenty of scope to develop both field-friendly screening tests as well as highly sensitive LRP assay suitable for a sophisticated laboratory, making TB diagnosis simple and cost effective on one hand and rapid and accurate on the other.

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Emerging Technologies for the Rapid Detection of Tuberculosis

Ruth McNerney

Prompt detection of tuberculosis is crucial, both to access appropriate treatment and to reduce transmission. Current tests are insensitive, slow or difficult to access and we urgently need affordable, robust 'real-time' tests that can be used at a health clinic for 'on-the-spot' diagnosis. In addition, there are as yet no effective tools for screening high-risk communities for infectious TB. In many countries control of the disease relies on passive case finding and sputum smear microscopy of self-presenting individuals with prolonged cough. While microbiology, immunology and molecular biology have provided some useful tools, they have not proved sufficient to control TB in poor-resource settings or those with a high prevalence of HIV. Attention has turned recently to alternative technologies. Several approaches are being investigated and while some tests are completely novel others have benefited from recent technological advances to improve their performance and accessibility.

One of the most interesting developments is a novel breathalyser-style test to assist with early detection of infectious respiratory cases (Rapid Biosensor Systems Ltd, UK). The test is aimed at detecting 'early TB' before the onset of severe, highly-symptomatic disease. Using a fully portable device, the test takes less than five minutes. Independent studies with a prototype instrument have demonstrated that little operator training is needed. Early data suggests that the test is capable of detecting TB in smear negative cases. Further evaluation is to be undertaken to determine the role of this technology in the control of tuberculosis.

The most promising of the new diagnostic technologies rely on the detection of biomarkers that predict active TB infection. However, a major bottleneck in TB testing is the preparation of specimens prior to analysis. A technology that avoids such problems is the analysis of volatile organic compounds (VOC) released by specimens. Samples of headspace gases taken from above specimens may be analysed or 'smelled' by olfactory sensing or through using 'artificial noses'. Detection of volatile biomarkers and recognition of complex patterns of compounds may be undertaken in minutes. An organisation in Tanzania (Apopo) is evaluating the use of trained 'sniffer' rats to screen sputum specimens for TB by their smell. They report that the method is considerably faster than microscopy, allowing a higher throughput of specimens in the laboratory[1]. A more technological approach involves electronic noses such as the Bloodhound™ (Scensive Technologies Limited, UK) where odours are exposed to a series of sensors that produce electronic signals when molecules bind. Software has been developed to analyse the binding patterns observed and to identify patterns predictive of TB[2]. The technology has the added advantage of very low running costs as the sampling does not consume any reagents. A more analytical approach can be taken by application of gas chromatography to identify components of the headspace gases. Recent advances in miniaturisation and the development of highly sensitive detectors promises to move this technology from the research laboratory into the field. Assessment of an ultra-rapid gas chromatograph, the zNose (Electronic Sensor Technology, USA), which incorporates a SAW (surface acoustic wave) sensor, is currently under way. Mass spectrometry is an alternative sensitive analytical tool that, when coupled to gas chromatography, provides highly specific chemical identification. Technological advances incurred through a space exploration programme are to be translated to the health field in a project recently funded by the Wellcome Trust. A simplified, robust mass spectrometer is to be transferred to Zimbabwe for evaluation for rapid diagnosis of pulmonary tuberculosis. Another interesting development is the application of mass spectrometry to the analysis of breath. When combined with sophisticated statistical analysis the data collected may be used to predict TB disease[3].

In conclusion, it would appear that alternative diagnostic technologies are emerging that may improve access to treatment and/or early detection of infectious cases. We should await their arrival and evaluation with some optimism.

Footnotes:

1 http://www.apopo.org

2 Fend, R. et al 2005 J Clin Microbiol 43:1745-51; Fend, R. et al. 2006 J Clin Microbiol. 44: 2039-45

3 Phillips, M. et al 2007. Tuberculosis 87: 44-52

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New Protocols for the Use of Lipid Biomarkers in the Rapid Detection of Tuberculosis

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Background: *Mycobacterium tuberculosis* synthesises a range of high molecular weight characteristic lipids not found in mammalian tissues. As reliable biomarkers for tuberculosis, the lipids with the greatest potential are the mycolic acids (MA) and phthiocerol dimycocerosate (PDIM) waxes. Sensitive methods have been developed for the detection of mycolic and mycocerosic acids in sputum and archaeological materials, using fluorescence high performance liquid chromatography (HPLC) and negative ion chemical ionisation (NI-CI) gas chromatography mass spectrometry (GC-MS) [1,2]. Updated protocols now allow the application of these sensitive methods to be completed within 48 to 36 hours.

Methods and Objectives: Experiments are in progress for the application of these new protocols for the sensitive detection of lipid biomarkers in sputum and blood samples from infected subjects. Alkaline hydrolysis releases phthiocerols and mycolic and mycocerosic acids. The latter are converted to pentafluorobenzyl (PFB) esters and the long-chain compounds are fractionated on silica gel cartridges. Mycocerosate PFBs are analysed by NI-CI GC-MS [1] and mycolate PFB esters are reacted with pyrenebutyric acid (PBA) and profiles recorded by fluorescence HPLC [2]. Having established the presence of the characteristic MA or PDIM lipid biomarkers, strenuous efforts will be made to drastically reduce the complexity and cost of the methods used to allow rapid and reliable detection within one day or less.

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NEHCRI: Strengthening TB Laboratory Services and Operational TB Research in Indonesia

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Background: The Novartis Institute for Tropical Diseases (NITD), Singapore, collaborated with two institutes from Indonesia (Eijkman Institute for Molecular Biology, Jakarta and Hasanuddin University, Makassar) to set up the NITD - Eijkman Institute - Hasanuddin University Clinical Research Initiative (NEHCRI). The aims of NEHCRI project are to be an effective partner to support the health care needs of the local population and to undertake clinical research in dengue fever, tuberculosis (TB) and malaria in Indonesia. NITD contributes broad scientific expertise, innovative technologies and financial support to improve the knowledge and skills of clinicians, scientists and health care workers working on those diseases. Two NEHCRI laboratories were set up this year to achieve the above-mentioned goals. One of the laboratories is situated at the Hasanuddin University hospital (Dr Wahidin Sudirohusodo hospital), Makassar, and the other one is at the Eijkman Institute, Jakarta.

Makassar is the capital city of South Sulawesi in Indonesia. Similar to other places in Indonesia, TB prevalence in Makassar has been increasing but the prevalence of drug- resistance is not known so far. Makassar does not have sufficient numbers of laboratories for performing TB culture and drug susceptibility tests (DST). Only one laboratory provides culture and DST for TB patients who can afford to buy its services (~20 USD).

Objectives: The aims of the TB NEHCRI laboratory is to strengthen TB laboratory services and undertake operartional research.

Strategic approach and Activities: The NEHCRI laboratory situated in Makassar has one room dedicated to TB diagnostics. The NEHCRI TB laboratory has been certified by the external certifier (Basler & Hofmann, Singapore) and the certifier recognised that the facility is built, equipped and operated in compliance with international state-of-the-art engineering rules and biosafety operating procedures. The laboratory is designated as a BSL-2+ and approved to use for diagnosing TB bacteria. The diagnostic activities are performed under supervision of the Central Laboratory of Dr Wahidin Sudirohusodo hospital. The diagnostic laboratory is run by three Indonesian staff who received the relevant training organised by NITD, National TB Programme (NTP) and its supranational laboratories (Institute of Medical and Veterinary Science, Adelaide and Thailand National Reference Laboratory, Tuberculosis Cluster Bureau of AIDS, TB and SITs, Bangkok, Thailand). NEHCRI TB laboratory is part of the 'Quality Assurance Laboratory Network' supervised by the NTP Indonesia and its supranational laboratories. In future, the NEHCRI TB laboratory may serve as a reference laboratory for South Sulawesi province.

The TB laboratory supports the diagnosis of TB and serves as a research laboratory. The laboratory receives samples from both Dr Wahidin Sudirohusodo hospital and the lung clinic close to the hospital. Smear microscopy, a liquid culture system (manual MGIT) and solid culture system have been routinely performed for diagnosing TB cases. DST is performed for all culture positive cases using manual MGIT. All TB diagnostic tools are made available free of charge for patients.

The availability of good quality diagnostic tools provides the basis for an epidemiology study that will determine the drug-resistance situation (MDR, XDR) and risk factors of TB (demographics, diabetes, HIV-TB co-infection). The epidemiology study will be complemented by molecular strain typing carried out at the NEHCRI laboratory, Eijkman Institute. In addition to the epidemiology study, scientists from NEHCRI and its affiliates will conduct operational research such as evaluating the usefulness of new rapid TB diagnostic tools in resource-limited settings.

It is a well-known fact that the lack of skilled personnel, especially in developing countries, currently jeopardises the laboratory component of TB control. Thus, strengthening human resource developments, capacity building and TB laboratory management are our priorities as well. NEHCRI staff will train staff from the hospital and the lung clinic in how to perform standardised culture methods and DST. We believe that the transfer of knowledge and technology will improve the quality of TB control in Makassar. The operational research activities and strengthening human resources are carried out in close collaboration with NTP and WHO Indonesia.

Conclusions: The availability of good quality TB culture and DST at the NEHCRI laboratory will help to improve TB case detection and case management in South Sulawesi. NITD's collaboration with Hasanuddin University and the Eijkman Institute will benefit not only the individual TB patients and their doctors but also provide the basic epidemiological data on TB in the region and improve the skill of laboratory staff. Our long term aspiration is that NEHCRI laboratories becomes one of the sites for carrying out clinical trials of promising new TB drugs/vaccines and diagnostic tools.

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Introducing IP-10 as a Specific Diagnostic Marker for Infection with M. Tuberculosis

Morten Ruhwald [1,2], Morten Bjerregaard-Andersen [3,4], Kristian Kofoed [1,2], Paulo Rabna [3], Jesper Eugen-Olsen [2], and Pernille Ravn [1]

Introduction: IFN-γ responses to M.Tuberculosis specific antigens (ESAT-6, CFP10 and TB7.7) are used in the in vitro diagnostic tests for tuberculosis. The in vitro tests have proven both sensitive and specific for latent and active tuberculosis disease, but may lead to false positive or negative diagnostic results as they rely on measurements of IFN-γ produced at very low levels.

We have screened a panel of 25 biomarkers as alternatives to IFN-γ as diagnostic markers of TB infection. This screening led to the discovery of one potentially highly interesting marker, the chemokine IP-10 (CXCL10). We here present data from a small-scale study in patients with active TB.

Material and methods: Twelve patients with active TB and positive IFN-γ test (Quantiferon); seven patients with active TB and negative IFN-γ test (of which five were immunosuppressed due to HIV+) and 11 healthy TB unexposed controls were included. Whole blood from each patient was set up in three cultures and stimulated with saline, TB specific antigens (ESAT-6, CFP10, TB7.7) and mitogen (PHA) respectively. Plasma was analysed by Luminex for IP-10, IL-2 and IFN-γ production and compared to IFN-γ measurement by Quantiferon ELISA.

Results: Concentration of IP-10 in plasma of whole blood was significantly higher in patients with active TB than in unexposed healthy individuals after in vitro stimulation with *M.tuberculosis* specific antigens. IP-10 was released in significantly higher amounts compared to IFN-γ and IL-2.

Of the seven patients with active TB but negative IFN-γ test, four produced strong IP-10 responses.

Discussion: We have identified a novel specific in vitro diagnostic marker for infection with M.tuberculosis which appears more sensitive than IFN-γ. As IP-10 is produced in significantly higher amounts compared to IFN-γ, a new highly sensitive test for the use in immunocompromised individuals, or a simple dipstick method for use in a resource-poor setting may be developed.

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Development of a Patch Test for the Diagnosis of Active Tuberculosis

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Background: The TB Patch consists of a protein that is applied to the skin of the forearm on the gauze of a band aid. The protein, MPT64, is produced only by actively-replicating members of the *M. tuberculosis* complex. Delivery of MPB64 by this method in clinical studies in the Philippines demonstrated an overall sensitivity of 94% for smear positive cases of TB, while eliciting no response in any TB symptom-free individuals (healthy controls), most of whom were PPD-positive.

The original clinical trials of the TB Patch used MPB64 purified from the spent medium of BCG Tokyo vaccine production, one of the few BCG strains that contains the gene. Production by this method is not feasible for a commercial product: yield was 0.05 mg/L, and purification required four chromatographic steps. We developed a method to produce recombinant MPT64 (rMPT64) and performed a dose-finding clinical evaluation of a TB Patch using the rMPT64 in Cape Town, RSA.

Objectives:

1. To develop a method of production of MPT64 that is commercially viable;
2. To perform a dose-finding study to estimate the safety, sensitivity and specificity of the TB Patch with rMPT64.

Methods: We cloned the MPT64 gene into an *E. coli* expression vector that over-expresses the protein and allows two chromatographic steps for purification. The TB Patch containing rMPT64 was tested on AFB-smear positive patients with TB, as well as on healthy controls with no signs or symptoms of TB. Doses of recombinant rMPT64 ranged from 75 to 600 ug.

Findings: We expressed rMPT64 in *E. coli* with high yield (250 mg/L) and purified the protein with two simple chromatographic steps as a recombinant untagged rMPT64. This rMPT64 elicited an immune response comparable to that of the native protein in a guinea pig model of active TB. We developed analytical release assays and performed scale-up optimisation to produce the protein by Good Manufacturing Practices (GMP).

We tested the TB Patch using several doses of the rMPT64 protein at clinics in Cape Town, RSA. These studies were conducted to select an optimal dose for administration of the rMPT64, since studies in the Philippines with the purified MPT64 showed optimal reactivity at 75 ug. Reactions to the rMPT64 were dose related and sensitivity of the test increased with increasing dose. Specificity was very high for all doses and the best sensitivity with rMPT64 was at the 600 ug dose: at this dose, sensitivity was 63%, and specificity was 91%.

Conclusions: The rMPT64 TB Patch Test demonstrated its potential to separate individuals with active infection from those who were BCG-vaccinated or are PPD positive. That rMPT64 was required at higher doses and was not as sensitive as the purified protein tested in the Philippines may relate to either formulation or properties of the recombinant protein itself that decreased potency. Analyses of purified and recombinant proteins are ongoing. Increasing the dose may also improve sensitivity.

The TB Patch Test is simple to apply, does not use sharps, and discriminates between actively replicating *M. tuberculosis* and latent TB infection/BCG vaccination. As a point-of-care screening tool for active TB, even with an unoptimised sensitivity of 63%, it holds great promise for affecting the TB epidemic by accelerating identification of TB disease in a population, without regard to site of infection. Preliminary evidence suggests that it can identify TB in HIV-infected individuals.

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Development of a US-based TB Laboratory Consortium for Mycobacterial Culture and DST in Response to Increased International Demand for Reference Laboratory Capacity

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Bacterial culture and DST is a critical component of diagnosis and treatment of drug-resistant TB. Currently, low income countries do not have adequate mycobacteriology laboratory infrastructure to deal with the growing prevalence of MDR-TB and XDR-TB. This is a critical gap in achieving WHO's global plan to scale-up treatment of drug-resistant TB to half of the global burden by 2015. One solution to this problem is to expand the role of US based Supranational TB Reference Laboratories (SRLs) through the development of a reference laboratory network, or consortium. This would allow for workload coordination, experience sharing, and cross-laboratory QC which would be beneficial for the US and developing countries. It could expand surveillance and development of novel approaches to tracking the emergence of resistance to SLDs. US based reference laboratories present an opportunity for accessing excess mycobacterial laboratory capacity in a country with declining rates of TB, while allowing US laboratories to maintain an appropriate testing repertoire and create opportunities for experienced mycobacteriologists. The MSLI, one of two US based SRL's, proposes the development of a New England based laboratory consortium that would make mycobacterial culture and DST available to projects in parts of the world without such services or in the process of developing appropriate laboratory capacity. The aim of this project is to build a global network that will eliminate unnecessary competition and duplication of effort, while expanding the available resources for DR-TB treatment scale-up.

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Towards Development of New Point-of-Patient-Care Tuberculosis Diagnostics

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Most cases of TB occur in countries where resources for health care are scarce and national health programmes struggle to find cost-effective ways of reducing transmission. The HIV pandemic has led to an increased diagnostic challenge as the sensitivity of smear microscopy is reduced further in TB-HIV co-infected individuals who commonly have smear negative pulmonary and extrapulmonary disease. Therefore there is an urgent need for new diagnostic tools.

Aim: To develop new cost-effective point-of-care TB diagnostics.

Design: Based on an ELISA technique (developed earlier by us) to measure LAM antigen secretion in urine (Scand J Infect Dis 33:279-284, 2001; Scand J Infect 34: 167-171, 2002) we have now developed a simpler dip-stick urine test and evaluated it in 35 culture verified Estonian TB patients and 15 healthy Swedish controls.

Results: 77% of the TB patients were correctly identified by the dip-stick test and all controls were negative.

Conclusions: Although promising, further increase in sensivity is needed and current work on this and a new immuno-chromatographic method will be presented.

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Application of Differential Mobility Spectrometry for Point-of-Care Diagnosis of Pulmonary Tuberculosis

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Background: The growing incidence of TB due to HIV/AIDS and the emergence of multidrug and extremely resistant strains have emphasised the need for improved strategies to control Mycobacterium tuberculosis (MTb). One of the major obstacles to the control of MTb has been the lack of a rapid and specific test for active pulmonary MTb. We have applied a novel sensor technology to detect volatile organic compounds (VOCs) from MTb to develop a point-of-care diagnostic device. The differential mobility spectrometer (DMS) is a highly sensitive mass analyser that has already been shown to be robust for field applications such as chemical and explosives detection. It is also relatively low cost and portable with a current working device weighing less than two pounds with integrated electronics.

Comparison	Growth Index (~bacilli/mL)	% Correct Classification	
		K-NN Algorithm	SVM Algorithm
TB Strain 1 vs. Media	10 (105)	81.25	87.5
TB Strain 1 vs. Media	500 (106)	93.3	86.7
TB Strain 1 vs. Media	900 (106-107)	100	100
TB Strain 1 vs. MAC	500	100	100
TB Strain 2 vs. Media	10	100	100
TB Strain 2 vs. Media	500	100	100
TB Strain 2 vs. Media	900	100	100
TB Strain 2 vs. MAC	500	100	100

Methods: Pure cultures of MTb were grown and the gaseous phase above the liquid (headspace) was collected using solid-phase microextraction (SPME). The compounds were then analysed with gas chromatography-mass spectrometry (GC-MS) in tandem with GC-DMS. MTb specific compounds were identified in comparison to matched media and control mycobacterium strains (M smegmatis, M avium) and their chemical structure was determined and confirmed with purified standards. In addition, the VOCs were analysed with the DMS and the ability to differentiate MTb from media or control strains was determined using signal processing and data analysis of recognised features.

Results: Using these methods, we have identified at least 15 VOCs that are associated with MTb growth. Most of these compounds were MTb-specific and were not released by *M smegmatis* or other bacteria studied. Using collected data along with novel algorithms, we have been able to distinguish TB from control mycobacterium and media with 81-100% classification accuracy (see table to the right) at concentrations in the lower limit of detection of BactecTM(~105 bacilli/ml). Optimisation of the DMS to detect the above compounds is under way in order to determine their presence in the breath of active pulmonary TB patients or to determine the lower limits of detection.

Conclusions: Our initial results suggest that these identified MTb VOCs are potential biomarkers for diagnosis of pulmonary MTb either in the breath of patients or the headspace of sputum. Both applications show promise for the development of a point-of-care device for detection of MTb or possibly rapid determination of MTb drug resistance.

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Rapid, Reliable and Easy Fluorometric Assay for Susceptibility Testing of Rifampicin in Mycobacterium Tuberculosis (FAST-RIF)

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Rationale: The evolution of multi-drug resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) threatens TB control world-wide. In order to combat this threat it is essential that rapid drug-susceptibly testing diagnostics are developed.

Objective: To develop a genotypic drug-susceptibly testing method that will enable the rapid diagnosis of rifampicin-resistance as a marker for MDR-TB and XDR-TB.

Methods: The rifampicin-resistance determining region (RRDR) of the rpoB gene from *Mycobacterium tuberculosis* was PCR amplified from either purified or crude DNA templates extracted from clinical isolates or the laboratory strain H37Rv. Subsequent mixing of the PCR amplicons and thermocycling allowed DNA duplexes to be formed between the RRDR's amplified from H37Rv and the respective clinical isolates. The thermal denaturation properties of the respective DNA duplexes were determined by measuring the amount of fluorescent dye bound to the DNA at different temperatures. The derivative of the intensity of fluorescence at different temperatures (dF/dT) was calculated. Drug-sensitive isolates were scored by the presence of a single peak (homoduplexes) within a defined temperature bin, while drug-resistant isolates were scored by the presence of two peaks (homoduplexes and heteroduplexes) within distinct temperature bins.

Results: In this study we describe a novel Fluorometric Assay for Susceptibility Testing of Rifampicin (FAST-RIF). Analysis of DNA extracted from 151 clinical isolates using the FAST-RIF method showed a sensitivity of 0.99 and a specificity of 1.00 for the detection of rifampicin-resistance when compared to the 'gold standard' routine culture based phenotyping method. The positive predictive value was 1.00 and the negative predictive value was 0.99. No statistical difference was detected in the performance of the method when applied to crude DNA from 131 boiled cultures, with initial results showing a sensitivity of 0.92 and a specificity of 0.95 when compared to the gold standard.

Conclusions: The FAST-RIF method allowed for the rapid, reliable and easy detection of genotypic rifampicin-resistance in *M. tuberculosis*. The versatility of the method allows for the adaptation to a single tube format as well as for the detection of non-synonymous single nucleotide polymorphisms conferring resistance to other anti-TB drugs.

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Performance of a T–Cell Based Assay for The Diagnosis of Tuberculosis in HIV-Infected Children

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Background:
Tuberculosis (TB) is a neglected disease in children; however, of the 8.3 million cases of active TB disease worldwide in 2000, almost 1 million occurred in children. The diagnosis of TB in children remains particularly problematic, especially in those co-infected with HIV due to difficulty in obtaining appropriate microbiological specimens, low yield of acid-fast staining of smear specimens resulting in delayed diagnosis, and poor sensitivity and specificity of tuberculin skin tests (TST). The discovery of Mycobacterium tuberculosis (MTB)-specific antigens has led to a significant new avenue for diagnosis. Promising enzyme-linked immunospot (ELISPOT) assays have been developed that detect in vitro production of interferon gamma (IFN-γ) by T cells in response to these antigens. However, there are limited data on the use of these assays for the detection of tuberculosis in young HIV-infected children.

Objectives

- To determine the sensitivity and specificity of ELISPOT in diagnosing active TB in young HIV-infected children.
- To assess the effect of age and the degree of immune suppression on IFN-γ secretion by T-cells in response to MTB-specific antigens.

Methods:
Three groups of children were recruited: HIV-infected children with suspected TB (n=101), HIV-infected children without TB (n=37) and HIV-uninfected children without TB (n=49).All children with suspected TB underwent a TST and chest X-ray and had at least 1 induced sputum specimen or 2 gastric washing specimens sent for TB microscopy and culture. All children diagnosed without TB were followed clinically to confirm this assessment. An ELISPOT assay was undertaken on all children, and HIV-infected children had a concomitant CD4 count. A final diagnosis of definite, probable, possible or not TB was assigned according to WHO criteria using clinical and microbiological evidence, and compared with results of the ELISPOT assay.

Results:
The median age of the 187 children was 20 months (IQR: 10 – 53). Definite or probable TB was diagnosed in 43% of the children with suspected TB. ELISPOT assays yielded determinate results in 93% of children, and were significantly more sensitive than TST for definite or probable TB (ELISPOT 71% (95% CI: 56 – 86) vs. TST 31% (95% CI: 14 – 48), p=0.0008). There was no significant difference in specificity between ELISPOT and TST. In contrast to TST where sensitivity was lower in those younger than 2 years of age (15%) or with severely immune suppression (CD4 < 15%) (0%), ELISPOT sensitivity was unimpaired by these factors and was 65% and 77% in each of these respective subgroups.

Conclusion:
The sensitivity of an ELISPOT assay for the diagnosis of TB in HIV infected children is reasonable (71%) and higher than TST. In contrast to TST, the performance of an ELISPOT assay appears relatively unimpaired by young age and severe HIV infection.

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Busisiwe Beko, Khayelitsha South Africa. MDR-TB counselor

They said I must cough the sputum and I said I have no sputum. I asked them to do an X-ray but they said they couldn't do it because I was pregnant. I told them that I wanted to sign the form so that I could have an X-ray. I was getting worse, getting thin and very weak. The X-ray showed I had TB. Now I was very, very worried because I had TB, HIV and I was pregnant. This was August 2005.

I started the TB treatment in September and in December I had to deliver my baby. I looked at the calendar and I thought: I am not going to make it, I will die before.

I made it and my baby was born on the 2nd of December 2005, but my TB treatment was not working. The doctor made a test and in February told me I had MDR-TB. I was so shocked because it was my first time to hear about this MDR. I knew nothing about it.

http://www.accessmed-msf.org/