

One day
symposium on
TB field
diagnostics



Abstracts

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	

Contents

Clinical, Immunological and Epidemiological Importance of Anti-Tuberculosis T cell Responses in HIV Infected Africans	1
Effect of HIV-1 Infection on T-Cell-based and Skin Test Detection of Tuberculosis Infection	2
Session 1: Current Field Diagnostics	
FIND Demonstration Projects: MGIT Culture and Drug Susceptibility Testing	5
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	6
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	7
Diagnostic Utility of LED Fluorescence Microscopy to Detect Acid-fast Bacilli in Sputum	9
Sputum Bleach-sedimentation Improves the Safety and Speed of Microscopy for Tuberculosis Diagnosis	10
Evaluation of the Capilia TB Assay and the GenoType Mycobacterium Assay to Identify MTB Complex Directly on Liquid Culture (MGIT)	11
Diagnostic Accuracy of the SH-HS Method for AFB Smears and Culture	12
The Colorimetric Indicator STC Accelerates Tuberculosis Culture Diagnosis	13
Comparison Between Lowenstein Jensen and MIGIT 960: Recovery and Time Rates	14
Optimisation of TB Field Testing: In-transit Sputum Decontamination & Culture on Colorimetric Selective Media for TB Diagnosis & Drug-susceptibility Testing	15
Improvement of Tuberculosis Case Detection and Reduction of Discrepancies Between Men and Women by Simple Sputum-submission Instructions: A Pragmatic Randomised Controlled Trial	17
7H9 Broth is an Ideal Tuberculosis Culture Medium for Resource-Limited Countries	19
Rapid Tests for Detecting MDR-TB in Kampala, Uganda	20
A Rapid Microcolony Susceptibility Test	21
Performance of Different Culture Systems for Isolation of TB and Implications for TB Control in High TB and HIV Endemic areas	22
Improving on Sputum Collection and Diagnosing Tuberculosis in the Field	23
Comparative Evaluation of BACTEC MGIT 960 System in The Gambia	24
Sensitive and Rapid Tuberculosis Culture Diagnosis with Disposable Filters Replacing the Laboratory Centrifuge	25
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	26
Monitoring Anti-tuberculosis Therapy with Fluorescein Diacetate (FDA) Microscopy Rapidly Determines Infectiousness and Screens for Drug Resistance	27
Comparative Evaluation of Mycobacteriophage Assay and Automated MGIT-960 Culture Method with a Novel ESAT-6 PCR Method for the Diagnosis of Tuberculosis	28
Comparative Analysis of Staining Methods for Mycobacterium Species	29
Cord formation: A Good Tool for Presumptive Identification of M.tuberculosis Complex.	30
Direct Detection of Rifampin Resistance in Mycobacterium Tuberculosis by the Nitrate Reductase Assay Applied Directly in Sputum Samples	31
Expedited Smear Microscopy Approach for the Diagnosis of Tuberculosis	32
Session 2: Operational Challenges for Field Diagnostics	
Challenges in TB Diagnostics in Secondary and Local Government Health Institutions in Ibadan, Nigeria	35
Evaluation of Fluorescence Microscopy for Diagnosis of Pulmonary Tuberculosis in a High HIV Prevalence Setting	37
Body Mass Index is More Reliable than Tuberculin Skin Testing for Diagnosing Adult Pulmonary Tuberculosis in Endemic regions	39

Multi-Drug Resistant Mycobacterium Tuberculosis (MDR-TB) in Ibadan, Nigeria: Challenges and Prospects	40
Diagnosing Abdominal Tuberculosis: A Retrospective Study from Nepal	42
Investing in People – The Importance of Quality Assurance in TB diagnostics in Developing Countries	44
High Level of Discordant Igra Results in HIV-infected Adults and Children	45
How Dangerous are Tests for Drug-resistant Tuberculosis?	46
Estimating the Resource Need for Using Culture to Diagnose Tuberculosis	47
Sputum Collection Centre Plays a Significant Role in TB control Programme in un-reached Tribal Areas of Orissa, India	48
T-SPOT.TB Offers No Advantage Over Tuberculin Skin Testing for Diagnosis of Tuberculosis in Young Children	49
In-house, Single-tube Nested Pcr for the Detection of TB in Children, Using Induced Sputum Samples	50
Poverty vis-à-vis TB Diagnostics. A Crisis for People Living with HIV/AIDS in Africa: Rethinking the Strategy for TB control	51
Gender Barriers to Tuberculosis Diagnosis	53
Establishing TB Culture Facility to Tackle the Challenge of MDR and XDR TB in the Kingdom of Lesotho	54
Sensitivity of QuantiFERON-TB Gold In-Tube in Zambian Adults with Smear Positive Tuberculosis	55
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	56
Treating Intestinal Helminths Augments Anti-mycobacterial Immunity, Converting Interferon-gamma Release Assay Diagnostic Tests for Tuberculosis Infection from Negative to Positive	58
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	61
The Mulago Inpatient Noninvasive Diagnosis of Pneumonia Study: A Platform for Investigating Novel TB Diagnostics	63
Low-cost Incubator Designs for Tuberculosis Culture Diagnosis in Resource-poor Areas	64
Molecular Beacons: Rapid Detection of Mycobacterium Tuberculosis and Drug Resistance in Specimens from Developing Countries	65
Enhanced Ex Vivo Stimulation of Mycobacterium Tuberculosis-Specific T cells in HIV-Infected Persons via Antigen Delivery by the Bordetella Pertussis Adenylate Cyclase Vector	66
Lam-ICT – Point of Care Test for Mycobacterial Infections	67
Pilot Study on the Efficacy of Beta Galactosidase Reporter Phage for Rapid Field Diagnosis of Tuberculosis from Sputum Samples	68
Optimised LRP Assay for TB Diagnosis	69
Emerging Technologies for the Rapid Detection of Tuberculosis	70
New Protocols for the Use of Lipid Biomarkers in the Rapid Detection of Tuberculosis	71
NEHCRI: Strengthening TB Laboratory Services and Operational TB Research in Indonesia	72
Introducing IP-10 as a Specific Diagnostic Marker for Infection with M. tuberculosis	74
Development of a Patch Test for the Diagnosis of Active Tuberculosis	75
Development of a US-based TB Laboratory Consortium for Mycobacterial Culture and DST in Response to Increased International Demand for Reference Laboratory Capacity	76
Towards Development of New Point-of-patient-care Tuberculosis Diagnostics	77
Application of Differential Mobility Spectrometry for Point-of-care Diagnosis of Pulmonary Tuberculosis	78
Rapid, Reliable and Easy Fluorometric Assay for Susceptibility Testing of Rifampicin in Mycobacterium Tuberculosis (FAST-RIF)	79
Performance of a T-Cell Based Assay for The Diagnosis of Tuberculosis in HIV-Infected Children	80

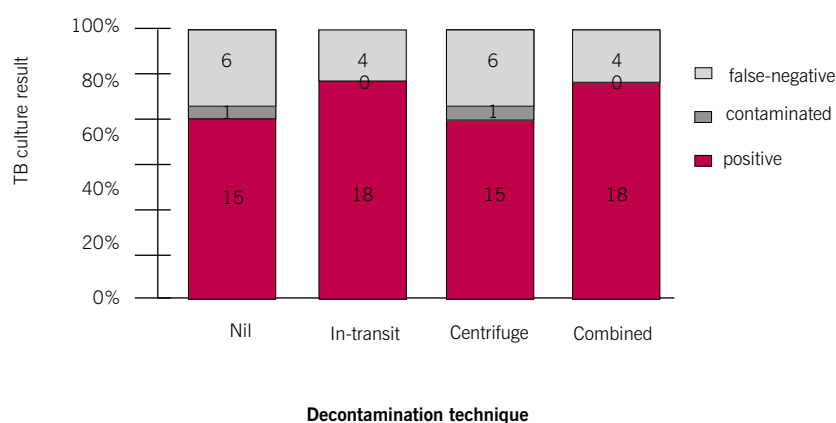
Optimisation of TB Field Testing: In-Transit Sputum Decontamination & Culture on Colorimetric Selective Media for TB Diagnosis & Drug-Susceptibility Testing

Beatriz Herrera¹, Eric Ramos¹, Robert H. Gilman^{1,2,3}, Louis Grandjean², Laura Martin², Jessica Alvarado^{1,2}, Willi Quino¹, Teresa Valencia¹, 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 Escombe⁴, 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 CO₂, 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

Selective - TLA cultures comparing decontamination techniques



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) & ciprofloxacin (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.

1 Lab. Investigación y Desarrollo, Universidad Peruana Cayetano Heredia, Perú 2 Asociación Benefica Prisma, Perú 3 Johns Hopkins Bloomberg School of Hygiene & Public Health, USA 4 Imperial College London, UK