One day symposium on TB field diagnostics

TB DYING FOR A TEST

Abstracts

CAMPAIGN FOR ACCESS TO ESSENTIAL MEDICINES

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

Session 3: Future Field Diagnostics

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

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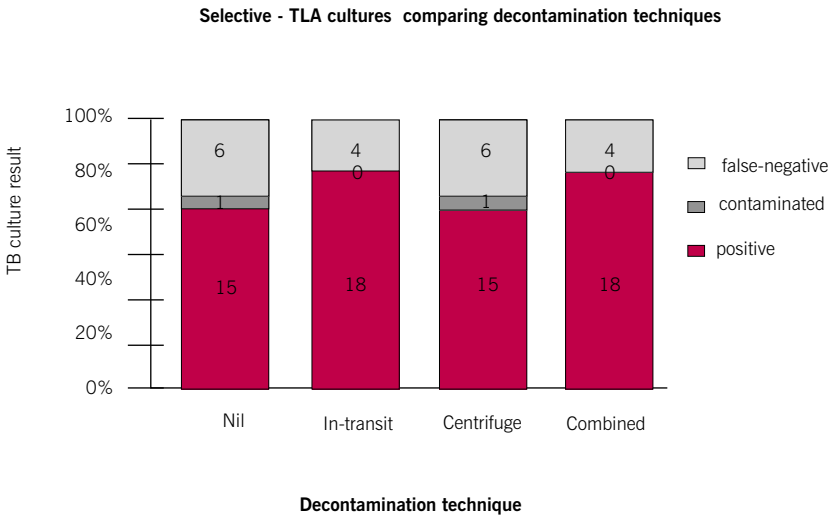
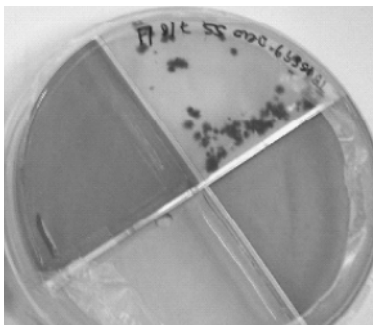
Background: TB particularly afflicts disadvantaged populations. Consequently, reference laboratories and the technologically demanding tests for MDRTB that they provide are least available to those in greatest need. In endemic settings, salivary micro-organisms usually overgrow sputum samples during 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) & 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.

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