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OPTIMIZATION OF TB FIELD TESTING: IN-TRANSIT SPUTUM DECONTAMINATION & CULTURE ON COLORIMETRIC SELECTIVE MEDIA FOR TB DIAGNOSIS & DRUG-SUSCEPTIBILITY TESTING

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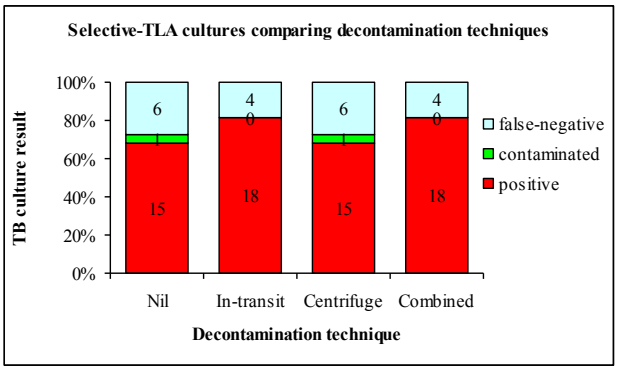
Background. TB particularly afflicts disadvantaged populations. Consequently, reference laboratories and the technologically demanding tests for MDRTB that they provide are least available to those in greatest need. In endemic settings, salivary micro-organisms usually overgrow sputum samples during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

Methods. Quantitative studies of TB colony numbers and time to growth were used to optimize 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 whilst 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) were 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 $C0_2$, 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 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



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 the TB was susceptible to them.

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.

Poster presentation at the *Medecins Sans Frontieres (MSF); Campaign for Access to Essential Medicines. Symposium on TB field Diagnostics 'Dying for a test'*. Cape Town, South Africa 7th November 2007.

ENHANCING TB CULTURE DIAGNOSIS WITH THE COLORIMETRIC INDICATOR STC

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Background. TB diagnosis by culture requires 3-8 weeks if mycobacterial growth is detected by naked eye. Examining cultures microscopically in the Microscopic-Observation Drug-Susceptibility (MODS) and Thin Layer Agar (TLA) techniques provides results in 1-3 weeks, but field practicality is limited by the time required to repeatedly examine cultures microscopically.

Objective. To evaluate the effect of a colorimetric indicator of mycobacterial growth on TB culture detection.

Methods. We investigated use of the redox indicator STC (2,3-diphenyl-5-(2-thienyl)tetrazolium chloride) in mycobacterial culture. In optimization experiments, serial dilutions of a laboratory strain of TB were cultured in liquid and solid media with and without STC. In clinical evaluation, 663 sputum samples were split and cultured in standard media that were examined microscopically, and in parallel in media containing STC that were examined by naked eye.

Results. In optimization experiments, STC produced coloration visible by naked eye and 50μg/ml did not inhibit growth, although 500μg/ml was inhibitory. In the clinical evaluation, 50μg/ml STC in broth (MODS) inoculated with clinical samples allowed naked eye detection of growth after a median of 12 days (IQR 9-14) vs. 10 days (IQR 8-14) microscopically. On solid media (TLA), detection took 14 days by both techniques (IQR 10-17 by microscopy vs. 12-18 by naked eye). Coloration also occurred with growth of bacterial or fungal contaminants, so a single microscopic examination of the sealed cultures was required for morphological confirmation of positive cultures. In all experiments, STC coloration was visible long before colonies could be seen by naked eye. Drug susceptibility was determined from parallel cultures containing isoniazid and rifampicin on the day of TB detection.

Conclusions. The slight delay between detection by microscope and by naked eye was offset by the considerable time saved by obviating repeated microscopic inspection of negative cultures. STC is an inexpensive and effective way to increase the efficiency and feasibility of rapid TB culture.

Late-breaker abstract presented by Scarlet Shell at the 2007 *American Society of Tropical Medicine & Hygiene annual conference* on November 4-8 in Philadelphia by Scarlet Shell as a poster presentation.

A SIMPLE COLOUR TEST FOR DIAGNOSING MDRTB & XDRTB UNDER FIELD CONDITIONS

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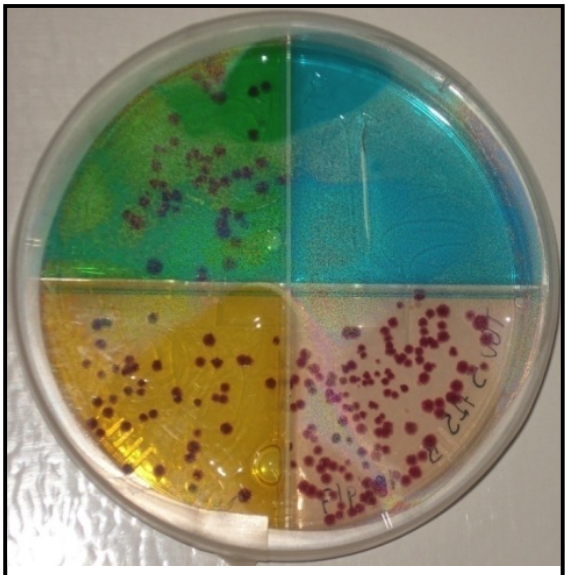
Funded by IFHAD: Innovation For Health And Development, The Sir Halley Stewart Trust, The Wellcome Trust and DFID: the Department For International Development of the British Government.

Background. The complexity and biohazard of conventional procedures largely restricts the use of TB culture and drug-susceptibility testing to reference laboratories. Thus sensitive TB, MDRTB and XDRTB diagnostics are infrequently available to patients in resource-poor settings who have greatest need for them.

Objective. To optimize safe sputum processing and culture for TB diagnosis and drug susceptibility testing for basic field laboratories in resource-poor settings.

Methods. Patients with suspected pulmonary TB expectorated into sputum pots containing disinfectant transport medium that liquefied and decontaminated sputum during transit. The sputum samples took between 6 hours and 5 days at room temperature to reach the laboratory. On arrival in the laboratory, the contents of the sputum pot were applied directly without any processing to each quadrant of a culture plate. The culture plate contained a transparent thin-layer of 7H11 agar made selective with antimicrobials to prevent contamination and also incorporated a colour-change indicator to detect positive cultures. Direct drug susceptibility testing for isoniazid, rifampicin and ciprofloxacin was also carried out concurrently in the other quadrants of the same culture plate. Immediately after sputum application, the culture plates were closed, double-sealed and incubated. Positive cultures were identified by naked-eye examination for colour change and *M. tuberculosis* was further confirmed by examining the areas of colour change within the sealed plates under a microscope with a 4x objective.

For comparison, the same patients expectorated another sputum sample into a normal dry pot that was processed by the gold-standard Centres for Disease Control reference laboratory technique utilising N-acetyl l-cysteine NaOH decontamination with centrifugation, vortex re-suspension and culture on 7H11 agar.



MDRTB growth (red dots) is visible in the detection (clear), isoniazid (green), & rifampicin containing (yellow) quadrants. Ciprofloxacin (blue) prevented TB growth, ruling out XDRTB.

Results. For 507 sputum samples cultured by both methods, 204 were culture-positive for *M.*

tuberculosis by one or both methods, 51% of which were also smear microscopy positive. The sensitivity of the novel test was 91%, significantly more sensitive than the conventional centrifuge decontamination method (76% diagnostic sensitivity, p=0.0001) and yielded significantly more colonies (p<0.0001). There was no significant difference between the tests in days to culture positivity (median 16 days; 75% positive within 3 weeks). Contamination resulted in the loss of 3.9% and 1.5% of test results, respectively (p=0.01). Processing 30 fresh sputa with the novel test took less than an hour, required less training and involved a similar sample processing bio-hazard to sputum smear microscopy, after which cultures were permanently sealed. In contrast, standard centrifuge decontamination took several hours, required specialized skills and risked biohazardous aerosol formation. The colour test materials cost ~$1 and utilized standard laboratory equipment (a normal incubator and a conventional microscope).

Conclusions. This simple colour-change technique allows safe, inexpensive and sensitive TB diagnosis with concurrent testing for MDRTB and screening for XDRTB in basic field-level laboratories. This has the potential to make modern TB diagnostics more widely accessible in resource-poor settings, increasing equity in TB diagnosis.

Poster presentation at CDC late-breaker session on TB *The 39th World Conference on Lung Health of the International Union Against TB & Lung Disease* Paris, October 20th 2008.

Background. The complexity and biohazard of conventional procedures for sputum liquefaction and decontamination largely restrict the use of TB culture with drug-susceptibility testing to reference laboratories. The complexity, advanced human capacity and expense of setting up and maintaining reference laboratories makes them a rare resource in the regions where tuberculosis is most common. Thus sensitive TB, MDRTB and XDRTB diagnostics are infrequently available to patients in resource-poor settings who have greatest need for them.

Objective. We aimed to optimize safe sputum processing and culture for TB diagnosis and drug susceptibility testing for basic field laboratories in resource-poor settings.

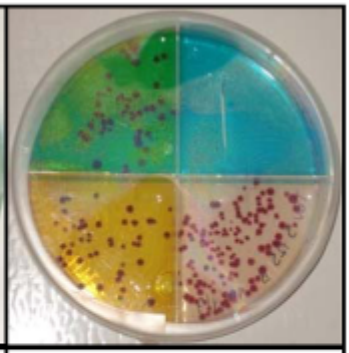
Strategy. In order to allow sensitive yet biosecure TB diagnostics in basic, regional laboratories we combined and refined a variety of sputum processing, culture, direct drug-susceptibility testing and tuberculosis detection techniques described by numerous researchers over the past several decades.

Participants. Patients with suspected pulmonary TB expectorated 385 sputa into pots containing disinfectant transport medium that liquefied and decontaminated sputum during transit. The sputum samples took between 6 hours and 5 days at room temperature to reach the laboratory.

Novel Colour Test technique. On arrival in the laboratory, the contents of the sputum pot were applied directly without any processing to each quadrant of a culture plate. The culture plate contained a transparent thin-layer of 7H11 agar made selective with antimicrobials to prevent contamination and also incorporated a colour-change indicator to detect positive cultures. Direct drug susceptibility testing for isoniazid, rifampicin and ciprofloxacin was also carried out concurrently in the other quadrants of the same culture plate. Immediately after sputum application, the culture plates were closed, double-sealed and incubated. Positive cultures were identified by naked-eye examination for colour change and *M. tuberculosis* was further confirmed by examining the areas of colour change within the sealed plates under a normal microscope with a low-power 4x objective.

A complete MDR-XDRTB colour test: sputum is collected into disinfectant transport medium that is applied directly to the culture plate as soon as it reaches the laboratory, without any processing.

An MDR-XDRTB colour test for drug-susceptible TB that only grew in the clear detection quadrant (red dots), not the coloured antibiotic-containing quadrants.



MDRTB grew in detection, isoniazid and rifampicin containing quadrants, but the quinolone (blue) prevented TB growth, ruling out XDRTB.

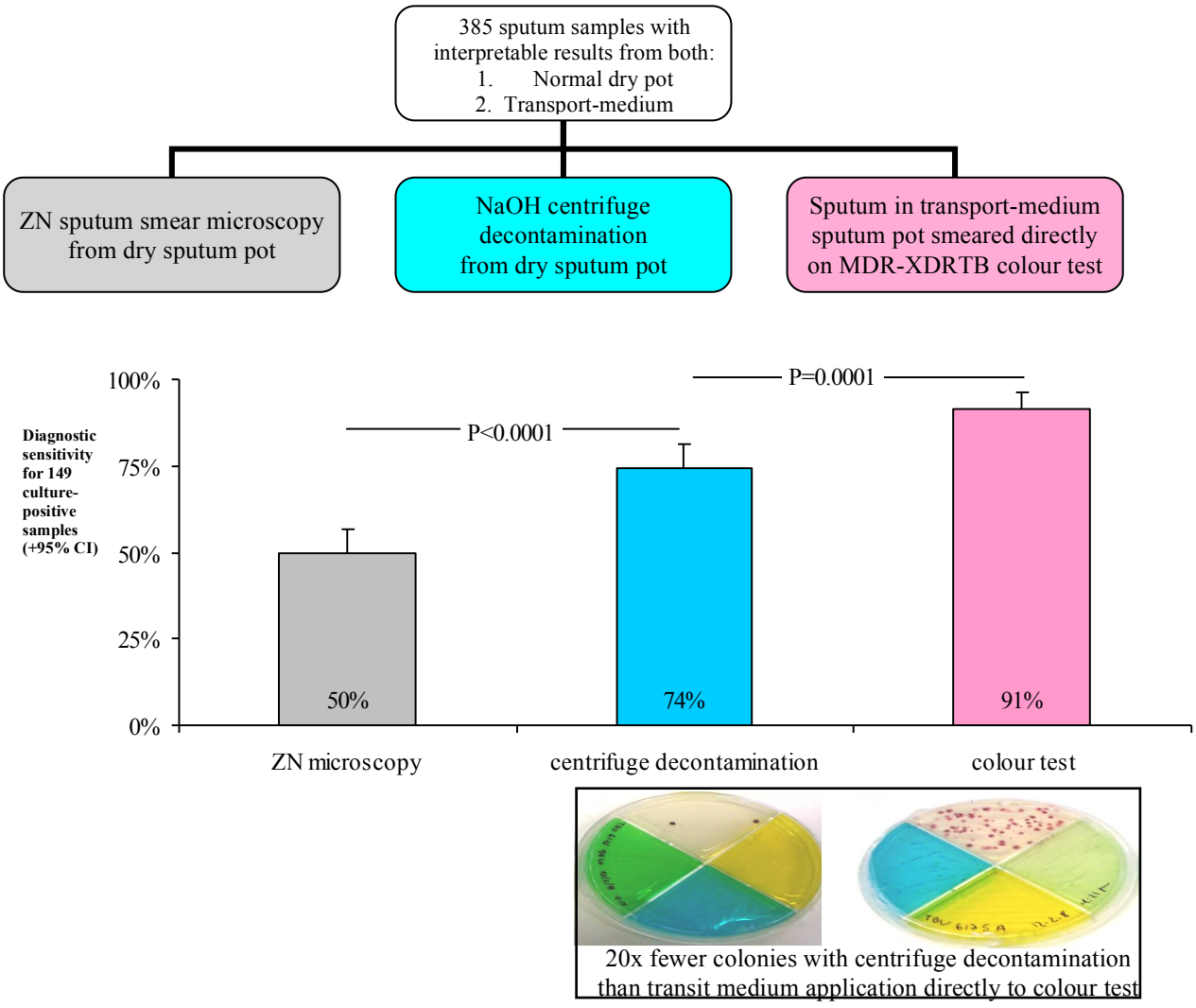
Control testing. For comparison, the same patients expectorated another sputum sample into a normal dry pot that was processed by the gold-standard Centres for Disease Control reference laboratory technique utilising N-acetyl l-cysteine NaOH decontamination with centrifugation, vortex re-suspension and culture on 7H11 agar.

Results. For 385 sputum samples cultured by both methods, 149 were culture-positive for *M. tuberculosis* by one or both methods, 49% of which were also smear microscopy positive. The gold standard test was culture-positivity by either test. The sensitivity of the novel test was 91%, significantly more sensitive than the conventional centrifuge decontamination method (74%

diagnostic sensitivity, p=0.0001) and yielded significantly more tuberculosis colonies (p<0.0001). The median estimated time to culture positivity with concurrent drug-susceptibility testing was 16 days, slightly faster than the centrifuge-decontamination method (p<0.01). Contamination resulted in the loss of 3.6% and 1.0% of test results, respectively (p=0.02).

Feasibility. Processing 30 fresh sputa with the novel test took less than an hour, required less training and involved a similar sample processing bio-hazard to sputum smear microscopy, after which cultures were permanently sealed. In contrast, standard centrifuge decontamination took several hours, required specialized skills and risked biohazardous aerosol formation. The colour test materials cost ~$1 and utilized standard laboratory equipment (a normal incubator and a conventional microscope).

Conclusions. This simple colour-change technique allowed safe, inexpensive and sensitive TB diagnosis with concurrent testing for MDRTB and screening for XDRTB in basic field-level laboratories. This has the potential to make modern TB diagnostics more widely accessible in resource-poor settings to increase equity in TB diagnosis.



Presentation by Carlton A Evans, Carlton.Evans@IFHAD.org

The collaborative work described here was carried out by Beatriz Herrera[1,2], Eric Ramos[1], Robert H. Gilman[1,2,3], Louis Grandjean[2], Laura Martin[2], Jessica Alvarado[1,2,], Teresa Valencia[1,2], Willi Quino[1], Gurjinder Sandhu[1,2], Jessica Alva[1,2], Rosario Sosa[1,2], Silvia Carrera[1,2], David Coleman[4], Denis Mitchison[4], Carlton A. Evans[1,2,3,5]

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Research funded by IFHAD: Innovation For Health And Development, The Sir Halley Stewart Trust, The Wellcome Trust and DFID: the Department For International Development of the British Government.

Invited oral presentation at FIND Scientific Forum on Recent Advances in TB Diagnostics, Paris 17th October, 2008 agenda at: at 10.35. Abstract published in the conference proceedings and summarized.

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]

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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).

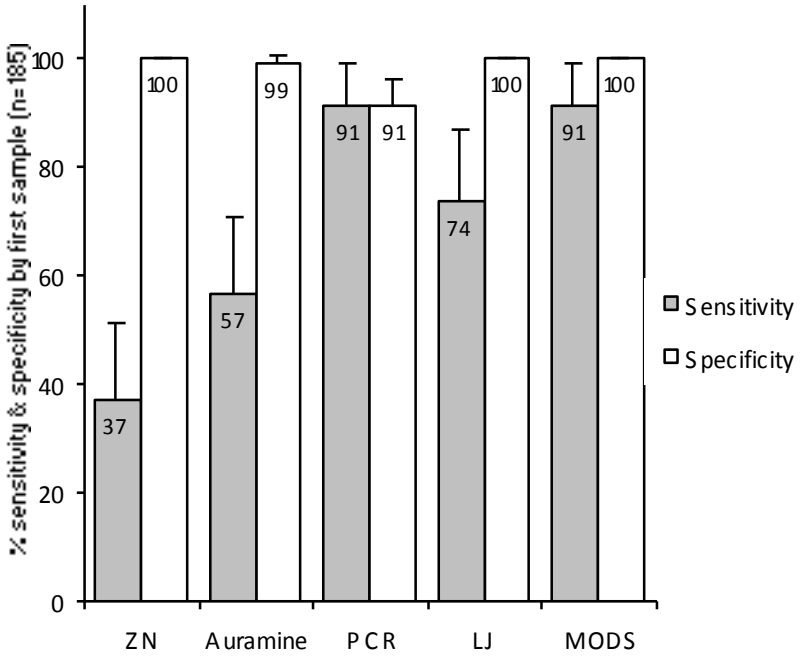
Subjects. 185 patients with symptoms suggestive of tuberculosis.

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

Methods. 185 patients with symptoms suggestive of tuberculosis underwent a standardized 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.

Poster presentation at the Medecins Sans Frontieres (MSF); Campaign for Access to Essential Medicines. Symposium on TB field Diagnostics 'Dying for a test'. Cape Town, South Africa 7th November 2007. Abstract published in the conference proceedings & http://www.msfaccess.org/fileadmin/user_upload/diseases/tuberculosis/DX_Abstacts_CT.pdf

VALIDATION OF THE MICROSCOPIC-OBSERVATION DRUG-SUSCEPTIBILITY (MODS) TECHNIQUE FOR DRUG-SUSCEPTIBILITY TESTING DURING TB THERAPY.

Marco Tovar [1,2], Teresa Valencia [1,2], Robert H Gilman [1,2,3], Lucy Caviedes [1,2,3,] Eric Ramos [1,2], Jessica Alvarado [1,2], Willi Quino [1,2], Beatriz Herrera [1,2], Carlton A Evans [1,2,4]

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2. Asociacion Benefica PRISMA, Lima, Peru
3. Johns Hopkins Bloomberg School of Public Health, Baltimore, USA
4. IFHAD: Innovation for Health and Development

Background. The Microscopic-Observation Drug-Susceptibility (MODS) technique has proven reliability for diagnosis and concurrent direct isoniazid and rifampicin drug-susceptibility testing for patients with suspected tuberculosis (TB). However, MODS has not been validated during TB treatment, when misleading mycobacterial sub-populations may be selected and when antimicrobials in sputum may confound in vitro direct drug-susceptibility testing. Consequently, patients whose sputum was collected after treatment commenced (or whose pre-treatment MODS assay failed) miss the opportunity for a rapid MODS drug-susceptibility test. We therefore assessed MODS reliability during TB treatment.

Methods. Sputum samples were collected from the same patients both prior to and during TB treatment. Sputa were tested with the MODS technique that provided direct concurrent isoniazid and rifampicin drug-susceptibility testing. Subsequently, the sub-cultured TB strain was tested with conventional indirect drug-susceptibility testing with the alamar blue and tetrazolium microplate assays. Results of these direct and indirect tests were compared with reference to the patients' phase of conventional first-line treatment.

Results. Paired direct MODS and indirect drug-susceptibility results were available for 1552 samples, 928 in the month prior to treatment, and 624 during therapy. TB therapy had no effect on the accuracy of direct drug-susceptibility testing relative to indirect testing. Specifically, the agreement between direct and indirect testing, respectively, was 92% vs. 91% for isoniazid susceptibility; 97% vs. 97% for rifampicin susceptibility; and 97% vs. 96% for MDRTB testing (all $P>0.1$). Treatment duration also had no effect on the level of agreement ($P>0.1$).

Conclusion. These results validate MODS drug-susceptibility testing during treatment. Thus, patients who have commenced TB therapy may be offered rapid drug-susceptibility testing with the MODS technique.

ABSTRACT *American J Tropical Medicine & Hygiene* 2009: 81(5); 212.
open access http://www.ajtmh.org/content/81/5_Suppl_1/201.full.pdf page 212
Prize oral presentation with young investigator scholarship (Abstract#1317, Presentation Number: 734) Session 125, Wednesday, November 10.15 – 12.00 am at the *The 58th annual conference of the American Society of Tropical Medicine & Hygiene* in Washington DC, USA 18-22nd Nov. 2009. [pdf]
http://www.ifhad.org/Abstracts/2009/ASTHM_pdfs/Tovar_MA_2009_p212_Microscopic-Observation_MODS_therapy_CAWE_zx.doc.pdf

RAPID DIRECT MDRTB TESTING BETTER PREDICTS CLINICAL OUTCOME THAN CONVENTIONAL DELAYED TESTING

MA Tovar,[1,2,5] T Valencia,[1,5] J Alvarado,[1,5] C Rojas,[1,5] L Caviedes,[1] SG Schumacher,[2,5] RH Gilman,[1,2,3] CA Evans[1,4,5]

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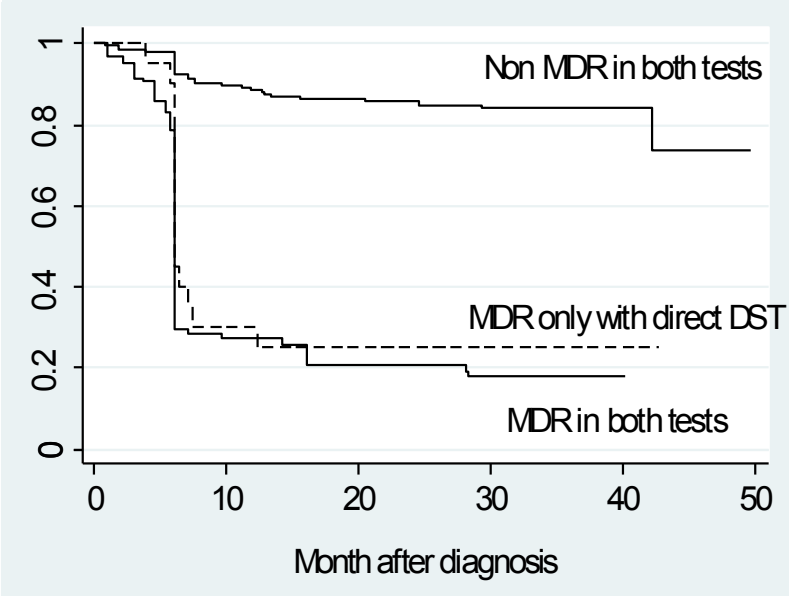
Background: Drug-susceptibility testing (DST) is an important tool in the control of multidrug-resistant tuberculosis (MDRTB). Conventional DST is done indirectly after first culturing the TB strain, thereby delaying the DST result. Newer DST tests are available that culture TB directly on antibiotic-containing media concurrently with drug-free culture. This leads to a much more rapid DST result, which may have an important positive impact on the patient's clinical outcome. Direct and indirect DST sometimes have discrepant results and we therefore investigated the clinical outcome of patients in relation to the agreement of results of both methods

Methods: Direct and indirect DST was performed on the same sputum sample (n=2,081) and the data were then grouped according to the concordance of the results (both tests MDR, both not MDR and discrepant results). Microscopic-Observation Drug-Susceptibility (MODS) was performed for direct DST and the tetrazolium microplate assay (TEMA) for indirect DST. For each pair of results we obtained the data on the patients' clinical condition at the end of TB treatment and the cured patients were followed-up for death or TB recurrence. We compared the risk of having a bad clinical outcome (failure, death or recurrent TB) for each group.

Results: Direct and indirect DST had consistent results in 97% of samples. Patients with an MDR result in direct DST and a non-MDR result in indirect DST had a hazard ratio of 7.3 (95% CI 4.2-12.8) of having a bad clinical outcome compared to the patients with no evidence of MDRTB. Patients found to be MDR in both methods had a hazard ratio of 2.9 (CI 95% 2.6 - 3.5) of having a bad clinical outcome compared to the patients with no evidence of MDRTB.



Conclusion: Patients diagnosed with MDRTB by direct DST but diagnosed to have non-MDRTB by indirect DST had the highest risk of having a bad clinical outcome. Thus, the results of rapid direct DST had greater clinical significance than slower indirect DST.

ABSTRACT *International Journal of TB and Lung Disease* 2010:14(11);S73.-74. Open access http://www.theunion.org/images/stories/journal/ABSTRACT_BOOK_2010_Web.pdf page 73. Poster discussion presentation at the International Union Against TB & Lung Disease 41st Annual Conference, Berlin, Germany, Nov 11-15, 2010. Abstract no. 010061.

VOLUME 14
NUMBER 11
NOVEMBER 2010
SUPPLEMENT 2

PAGES S1–S387
ISSN 1027 3719

The International Journal of Tuberculosis and Lung Disease

The Official Journal of the International Union Against Tuberculosis and Lung Disease

ABSTRACT BOOK

41st World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (The Union)

BERLIN · GERMANY
11–15 NOVEMBER 2010

PC-100618-13 Rapid direct MDR-TB testing better predicts clinical outcome than conventional delayed testing

M A Tovar,[1,2] T Valencia,[1] J Alvarado,[1] C Rojas,[1] L Caviedes,[1] S Schumacher,[2] R H Gilman,[1,2,3] C Evans.[1,4,5]
[1]Universidad Peruana Cayetano Heredia, San Martin de Porres, Lima, [2]Asociacion Benefica PRISMA, San Miguel, Lima, Peru; [3]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA; [4]Imperial College London, London, [5]Innovation for Health and Development, London, UK.
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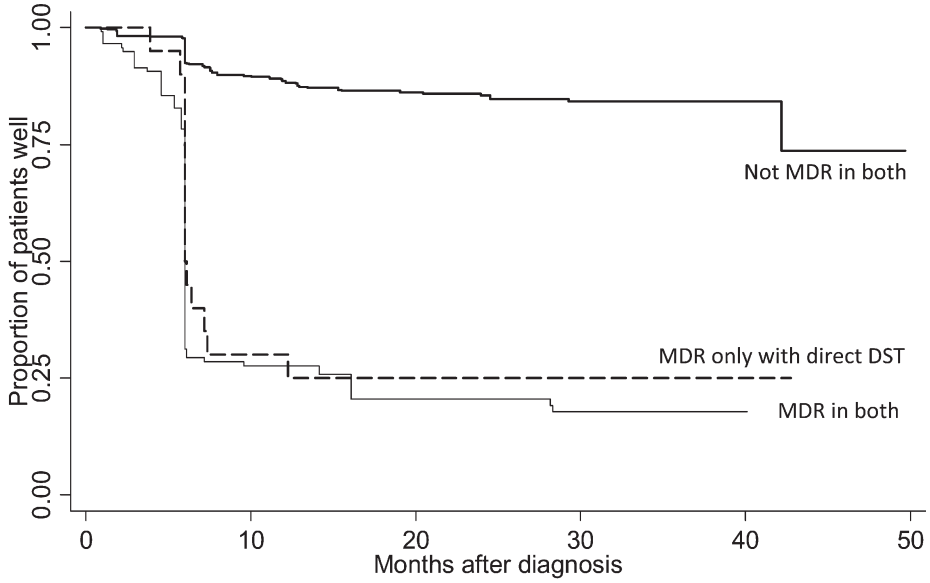
Background: Drug susceptibility testing (DST) is an important tool in the control of multidrug-resistant tuberculosis (MDR-TB). Conventional DST is done indirectly after first culturing the TB strain, thereby delaying the DST result. New DST tests are emerging that culture TB directly on antibiotic-containing media concurrently with drug-free culture. This leads to a much more rapid DST result, which may have an important positive impact on the patient's clinical outcome. Direct and indirect DST may have discrepant results and we therefore investigated the clinical

outcome of patients in relation to the agreement of results of both methods.

Methods: Direct and indirect DST was performed on the same sputum sample (n = 2081) and the data were then grouped according to the concordance of the results (both MDR, both not MDR or discrepant result). For each pair of results we obtained the data on the patients' clinical condition at the end of TB treatment and the cured patients were followed-up for the emergence of a new episode of TB. We compared the risk of having a bad clinical outcome (failure, death or recurrent TB) for each group.

Results: Direct and indirect DST had consistent results in 97% of samples. Patients with an MDR result in direct DST and a non-MDR result in indirect DST had a hazard ratio of 7.3 (95%CI 4.2–12.8) of having a bad clinical outcome compared to the patients with no evidence of MDR-TB. Patients found to be MDR in both methods had a hazard ratio of 2.9 (CI 95% 2.6–3.5) of having a bad clinical outcome compared to the patients with no evidence of MDR-TB.

Conclusion: Patients diagnosed with MDR-TB by direct DST but diagnosed to have non-MDR-TB by indirect DST had the highest risk of having a bad clinical outcome. Thus, these results of rapid direct DST had greater clinical significance than slower indirect DST.



OPERATIONAL EFFECTIVENESS OF SENSITIVE TB CULTURE AND DRUG-SUSCEPTIBILITY TESTING IN A HIGH-PREVALENCE SETTING

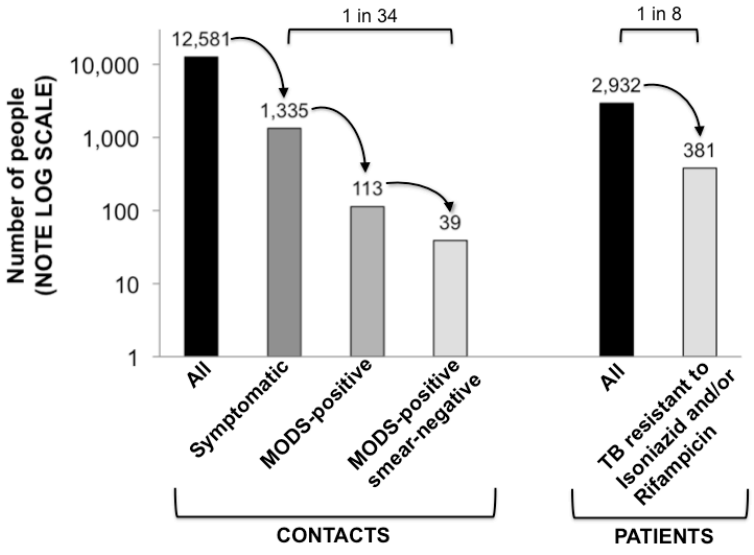
Samuel G. Schumacher[1,2], Teresa Valencia[3,4], Rosario Montoya[2,4], Marco Tovar[2,3] Arquimedes Gavino[2,3], Silvia Carrera[2,3,4], Robert H. Gilman[2,3,5], Carlton A. Evans[1,3,6]

[1]Innovation For Health And Development, UK ; [2]Asociación Benefica Prisma, Perú; [3]Laboratorio de Investigación y Desarrollo, Universidad Peruana Cayetano Heredia, Perú; [4]ADRA Peru, Lima, Peru; 5Johns Hopkins Bloomberg School of Hygiene and Public Health, USA; [6]Imperial College London, UK.

Background: Liquid culture systems are available for TB testing that provide higher sensitivity than Ziehl Neelson (ZN) microscopy and allow rapid concurrent testing for drug-resistance. Their scale-up is recommended in low- and middle-income countries but data on their effectiveness under operational conditions is scant.

Setting: Shantytown with 500,000-person population in Northern Lima, Peru that principally utilized ZN microscopy for TB diagnosis from 2002-2010.

Methods: The high number of TB suspects (>100 daily) did not allow culture testing of every sample therefore culture was focused on two high-risk groups: TB patients and their symptomatic contacts. 4,267 Drug-resistance Microscopic-Observation Susceptibility (MODS) cultures were performed for TB detection and concurrent drug-resistance testing.



Results: MODS testing of 2,932 TB patients determined that 381 (13%) had drug-resistant TB (DRTB; i.e. resistant to Isoniazid, Rifampicin or both), so 7.7 patients needed to have MODS culture per drug-resistant case identified (16 per MDR case). Drug resistance was more common among re-treatment cases (26%; risk ratio=1.9, P<0.001; population attributable fraction: 16%). Thus, restricting MODS testing to the 22% of TB patients who were re-treatment cases would only detect 35% of all DRTB (39% of MDRTB). Additionally among 12,581 contacts, 1,335 (11%) had respiratory symptoms, 113 of whom were MODS culture positive (i.e. 0.9% of all contacts and 8.5% of symptomatic contacts). Of these culture-positive contacts 39 (35%) were ZN microscopy-negative. Thus 34 symptomatic household contacts needed to have MODS culture per smear-negative TB case identified.

Conclusion: In operational settings, modern diagnostic tests that offer sensitive diagnosis and rapid DRTB testing may provide greatest public health impact when focused on TB patients rather than on enhanced case-finding. These calculations may be adjusted for settings with different rates of smear-negative TB and DRTB.

ABSTRACT Presented at the International Union Against TB & Lung Disease 41st Conference in Berlin, Germany, Nov 11-15, 2010. Abstract # 0100042.

VOLUME 14
NUMBER 11
NOVEMBER 2010
SUPPLEMENT 2

PAGES S1–S387
ISSN 1027 3719

The International Journal of Tuberculosis and Lung Disease

The Official Journal of the International Union Against Tuberculosis and Lung Disease

ABSTRACT BOOK

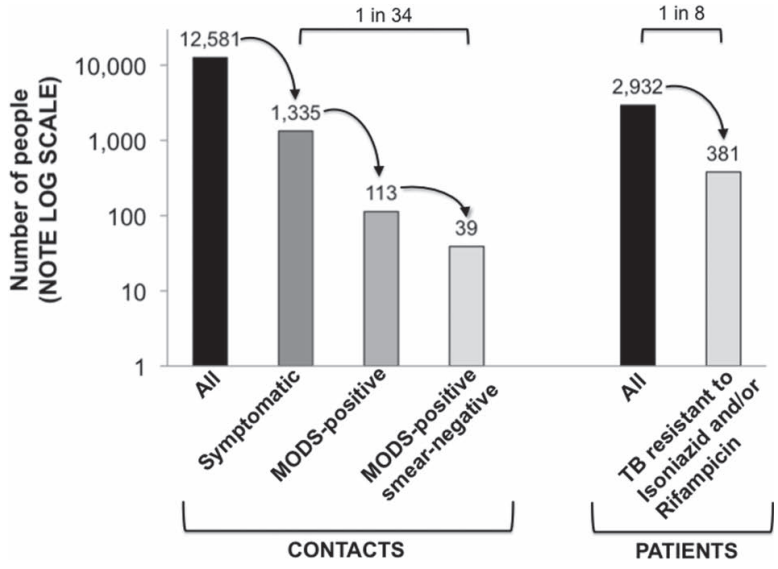
41st World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (The Union)

BERLIN · GERMANY
11–15 NOVEMBER 2010

PC-100042-13 Operational effectiveness of TB culture and drug susceptibility testing in a high-prevalence setting

S G Schumacher,[1,2] T Valencia,[3,4] R Montoya,[2,4] M Tovar,[2,3] A Gavino,[2,3] S Carrera,[2,3,4] R H Gilman,[2,3,5] C A Evans.[1,3,6]
[1]IFHAD: Innovation For Health And Development, London, UK; [2]Asociación Benefica Prisma, Lima, [3]Laboratorio de Investigación y Desarrollo, Universidad Peruana Cayetano Heredia, Lima, [4]ADRA Peru, Lima, Peru; [5]Johns Hopkins Bloomberg School of Hygiene and Public Health, Baltimore, MD, USA; [6]Imperial College London, London, UK.
e-mail: schumacher.samuel@gmail.com

Background: Liquid culture systems are available for TB testing that provide higher sensitivity than Ziehl-Neelson (ZN) microscopy and allow rapid concurrent testing for drug-resistance. Their scale-up is recommended in low- and middle-income countries but data on their effectiveness under operational conditions is scant.
Setting: Shantytown with 500 000-person population in Northern Lima, Peru that principally utilized ZN microscopy for TB diagnosis from 2002–2010.
Methods: The high number of TB suspects (>100 daily) did not allow culture testing of every sample therefore culture was focused on two high-risk groups: TB patients and their symptomatic contacts. 4267 Microscopic-Observation Drug-Susceptibility (MODS) cultures were performed for TB detection and concurrent drug-resistance testing.



Results: MODS testing of 2932 TB patients determined that 381 (13%) had drug-resistant TB (DRTB; i.e. resistant to isoniazid, rifampicin or both), so 7.7 patients needed to have MODS culture per drug-resistant case identified (16 per MDR case). Drug resistance was more common among re-treatment cases (26%; risk ratio = 1.9, $P < 0.001$; population attributable fraction: 16%). Thus, restricting MODS testing to the 22% of TB patients who were re-treatment cases would only detect 35% of all DRTB (39% of MDR-TB). Additionally among 12 581 contacts, 1335 (11%) had respiratory symptoms, 113 of whom were MODS culture positive (i.e., 0.9% of all contacts and 8.5% of symptomatic contacts). Of these culture-positive contacts 39 (35%) were ZN microscopy-negative. Thus 34 symptomatic household contacts needed to have MODS culture per smear-negative TB case identified.

Conclusion: In operational settings, modern diagnostic tests that offer sensitive diagnosis and rapid DRTB testing may provide greatest public health impact when focused on TB patients rather than on enhanced case-finding. These calculations may be adjusted for settings with different rates of smear-negative TB and DRTB.

A SIMPLE COLOUR TEST FOR DIAGNOSING MDRTB & XDRTB UNDER FIELD CONDITIONS

MA Tovar,[1,2] C Osorio,[1] E Ramos,[1] M Llacza,[1] B Herrera,[1] SG Schumacher,[2] RH Gilman,[1,2,3] CA Evans[1,4,5]

[1]Universidad Peruana Cayetano Heredia, San Martin de Porres, Lima, Peru
[2]Asociación Benefica Prisma, San Miguel, Lima, Peru
[3]John Hopkins Bloomberg School of Public Healths, Baltimore, United States of America
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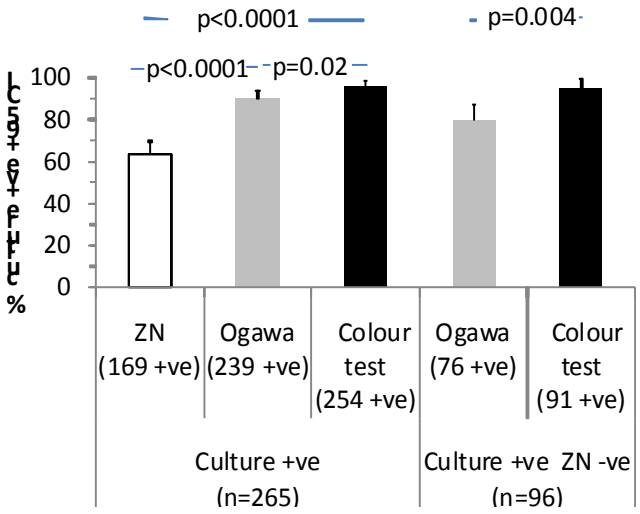
Background: Increasing rates of drug-resistant and smear-negative TB require improved TB diagnostics that are easy to use, affordable and equipment-minimal. The MDR/XDRTB Colour Test is a simple, Thin-Layer-Agar-based non-proprietary culture technique that offers concurrent MDR testing and XDR screening. We compared its performance with a standard culture technique.
Methods: Sputum samples were collected from patients with suspected TB (n= 788). Smear microscopy was done directly from sputum samples, which subsequently underwent culture with the Peruvian standard protocol of modified-Petroff NaOH-decontamination and culture on Ogawa medium. Samples for the Colour Test were collected in parallel directly into sputum pots containing disinfectant that decontaminated the sputum in-transit. Upon arrival in the lab, these were directly applied to selective culture medium without any processing. Positive cultures were indicated by colour change.
Results: 265 samples had a culture-positive result and both Colour Test and Petroff-Ogawa were significantly more sensitive than ZN microscopy (96% and 90% vs. 63%; p<0.0001). The Colour Test also had higher sensitivity than Petroff-Ogawa (p=0.02) and this difference was greatest in the 128 smear-negative samples that were culture-positive (95% vs. 79%; p=0.002). Results were obtained more rapidly in the Colour Test with similar (17 vs. 21 days; p<0.0001) vs. 2.4%; contamination rates (3.5% detected p=0.2). The Colour Test all culture-drug-resistant TB in 18% of day as positive samples the same agreement culture detection with 99% with delayed indirect testing.



Conclusion: The Colour Test is a simple culture technique that offers superior characteristics over the current standard culture method. It addresses the rising challenges of drug-paucibacillary disease and resistance while using simple and effective technology that is appropriate to resource-poor settings were these problems are most common.

ABSTRACT Presented at the International Union Against TB & Lung Disease 41st Annual Conference in Berlin, Germany, November 11-15, 2010. Abstract # 0101296.

VOLUME 14
NUMBER 11
NOVEMBER 2010
SUPPLEMENT 2

PAGES S1–S387
ISSN 1027 3719

The International Journal of Tuberculosis and Lung Disease

The Official Journal of the International Union Against Tuberculosis and Lung Disease

ABSTRACT BOOK

41st World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (The Union)

BERLIN · GERMANY
11–15 NOVEMBER 2010

PC-101296-13 A simple colour test for diagnosing MDR-TB and XDR-TB under field conditions

M Tovar,[1,2] S Schumacher,[2] C Osorio,[1] E Ramos,[1] M Llacza,[1] B Herrera,[1] R H Gilman,[1,2,3] C A Evans.[1,4,5] [1]Universidad Peruana Cayetano Heredia, San Martin de Porres, Lima, [2]Asociación Benefica Prisma, San Miguel, Lima, Peru; [3]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA; [4]Imperial College London, London, [5]Innovation for Health and Development, London, London, UK.
e-mail: 03195@upch.edu.pe

Background: Increasing rates of drug-resistant and smear-negative TB require improved TB diagnostics that are easy to use, affordable and equipment-minimal. The MDR/XDR-TB Colour Test is a simple, Thin-Layer-Agar-based non-proprietary culture technique that offers concurrent MDR testing and XDR screening. We compared its performance with a standard culture technique.
Methods: Sputum samples were collected from patients with suspected TB ($n = 788$). Smear microscopy was done directly from sputum samples, which subsequently underwent culture with the Peruvian standard protocol of modified-Petroff NaOH-decontamination and culture on Ogawa medium. Samples for the Colour Test were collected in parallel directly into sputum pots containing disinfectant that decontaminated the sputum in-transit. Upon arrival in the lab, these were directly applied to selective culture medium without any processing. Positive cultures were indicated by colour change.
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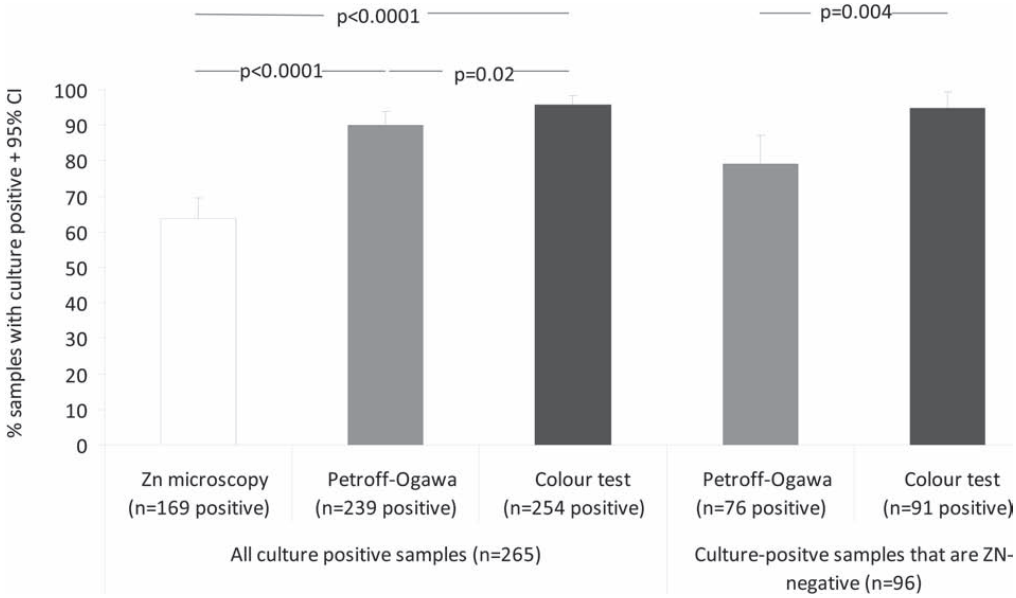


both Colour Test and Petroff-Ogawa were significantly more sensitive than ZN microscopy (96% and 90% vs. 63%; $P < 0.0001$). The Colour Test also had higher sensitivity than Petroff-Ogawa ($P = 0.02$) and this difference was greatest in the 128 smear-negative samples that were culture-positive (95% vs. 79%; $P = 0.002$). Results were obtained more rapidly in the Colour Test (17 vs. 21 days; $P < 0.0001$) with similar contamination rates (3.5% vs. 2.4%; $P = 0.2$). The Colour Test detected drug-resistant TB in 18% of all culture-positive samples the same day as culture detection with 99% agreement with delayed indirect testing.

Conclusion: The Colour Test is a simple culture technique that offers superior characteristics over the current standard culture method. It addresses the rising challenges of paucibacillary disease and drug-resistance while using simple and effective technology that is appropriate to resource-poor settings where these problems are most common.

COMPARISON OF TB DIAGNOSIS WITH THE MDR/XDRTB COLOUR TEST & MODS

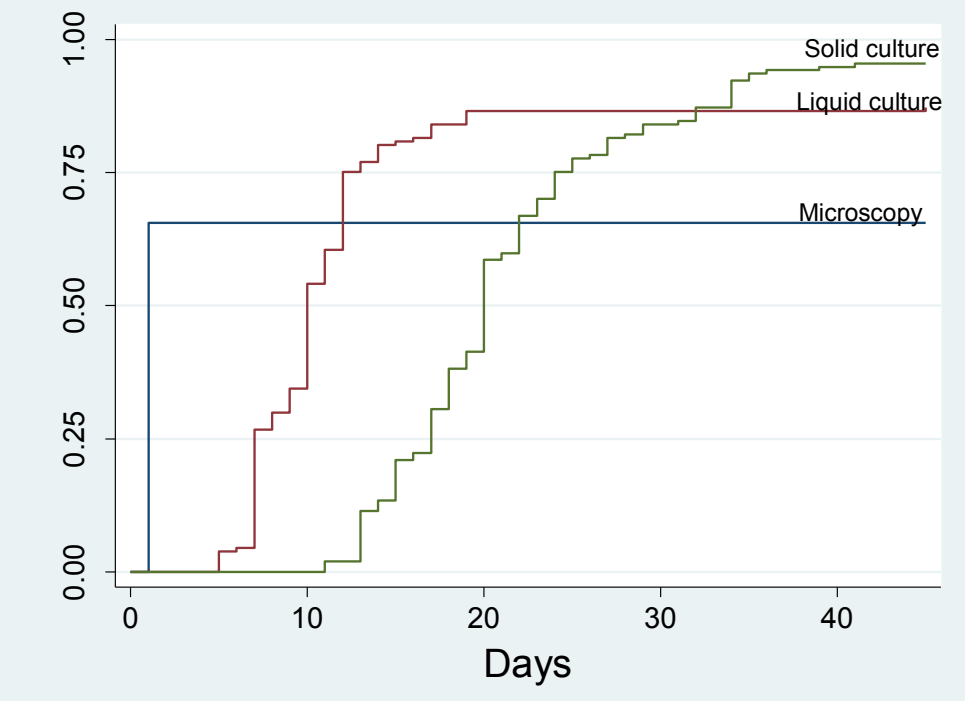
MA Tovar[1,2], SG Schumacher[1,2], C Osorio[1,2], E Ramos [1,2,3], M Llacza[1,2,3], T Valencia[1,2], B Herrera[1,2], CA Evans[1,2,4]
1. IFHAD: Innovation For Health And Development, London, UK; 2. Universidad Peruana Cayetano Heredia, Lima, Peru; 3. Asociación Benéfica Prisma, Lima, Peru; 4. Imperial College London, UK

Background: The diagnosis of microscopy-negative TB and MDRTB may be achieved by non-commercial solid or liquid-culture techniques that we compared in Peru.

Methods: Adults (n=544) with suspected TB provided sputum that was split between:
(1) un-concentrated Ziehl-Neelsen sputum smear microscopy;
(2) solid-culture: the MDR/XDRTB Colour Test with disinfection in the sputum pot, the contents of which were applied directly to thin-layer agar that was sealed, incubated and changed colour if micro-organisms grew; and
(3) liquid-culture: the MODS technique that decontaminated sputum with NaOH and centrifugation, re-suspended in broth, incubated and screened microscopically for TB growth.

Results: Fewer solid than liquid-cultures failed due to indeterminate or contaminated results (6/544, 1.1% versus 77/544, 14%, P<0.0001). Of 168 culture-positive samples with fully interpretable results, 156 (93%) were positive by solid-culture vs 164 (98%) by liquid-culture (P=0.08). Of 189 culture-positive samples, 173 (92%) were positive in solid-culture vs 165 (87%) in liquid-culture (P=0.2). Microscopy detected 125 (66%) of the culture-positive samples, less than either culture technique (P<0.0001). Microscopy was most rapid; positive liquid-cultures were detected in median 10 days, faster than 20 days for solid-cultures (P<0.0001). Solid and liquid-culture MDRTB-testing had 97% agreement. A technician working full-time managed ~60 microscopies/day, ~30 solid-cultures or ~10 liquid-cultures. Costs were lowest for microscopy, higher for solid-culture and highest for liquid-culture.

Conclusions: Smear microscopy is rapid, inexpensive but insensitive and cannot test for MDRTB. Solid-culture was simple, quick to perform and had greater biosafety than liquid-culture, which required greater resources. Liquid-culture provided results more rapidly but failed more often; sensitivities were similar. These results inform the selection of appropriate diagnostic techniques for different settings.



ABSTRACT Poster discussion presentation PC-1270-29 in session 34. Rapid TB diagnosis II at the International Union Against TB & Lung Disease 42nd Annual Conference in Lille, France, October 26-30, 2011. Category: Scientific research Topic/Subtopic: B02: Clinical research, treatment & care / TB diagnostic: culture & rapid detection methods.

Comparación del diagnostico de la TB mediante MDR/XDRTB Color test y MODS

MA Tovar,[1,2] SG Schumacher,[1,2] C Osorio, [1,2] E Ramos, [1,2,3] M Llacza, [1,2,3] T Valencia, [1,2] B Herrera, [1,2] CA Evans[1,2,4]

1. IFHAD: Innovation For Health And Development, London, UK. 2. Universidad Peruana Cayetano Heredia, Lima, Perú; 3. Asociación Benéfica Prisma, Lima, Perú; 4. Imperial College London, UK.

Introducción: El diagnostico de la TB baciloscopia negativa y la TB MDR pueden ser hechas por técnicas de cultivo no comerciales en medios solido o liquido los cuales comparamos en Perú.

Métodos: A adultos (n=544) con sospecha de TB dieron una muestra de esputo la cual fue dividida para:

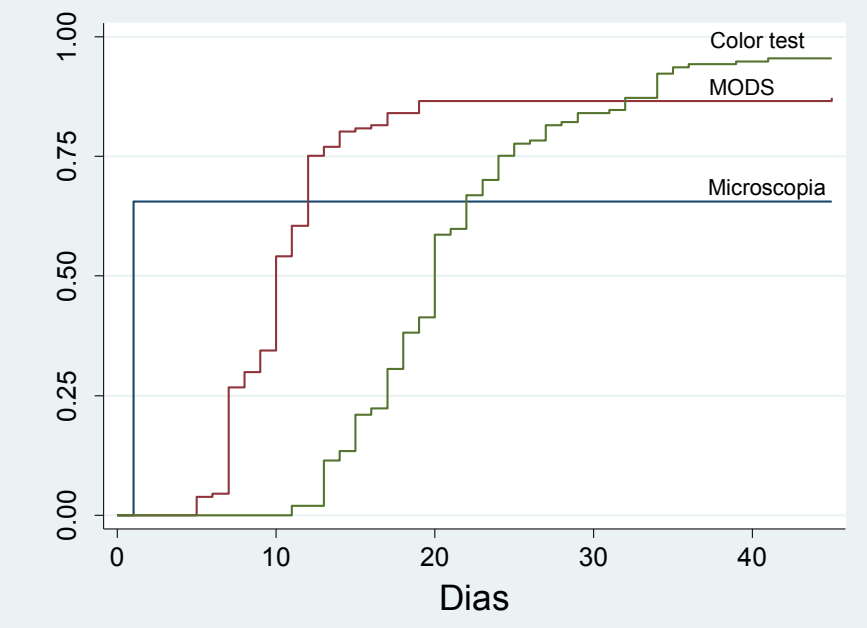
(1) microscopia con tinción de esputo Ziehl-Neelsen;

(2) cultivo en medio solido: MDR/XDRTB Colour Test con desinfección en el frasco seco de esputo, el contenido del cual se aplico directamente al agar de capa delgada la cual fue sellada, incubada y cambio de color si hubo crecimiento de microorganismos; y

(3) cultivo en medio liquido: MODS con decontaminación del esputo con NaOH y centrifugación, re-suspendido en un caldo, incubado y buscando microscópicamente el crecimiento de TB.

Resultados: Menos cultivos en medio solido fallaron que cultivos en medios líquidos debido a resultados indeterminados o contaminados (6/544, 1.1% versus 77/544, 14%, p<0.0001). De 168 cultivos con resultados interpretables, 156 (93%) fueron positivos en medio de cultivo solido vs 164 (98%) en cultivo liquido (p=0.08). De 189 cultivos positivos, 173 (92%) fueron positives en medio de cultivo solido vs 165 (87%) cultivo liquido (p=0.2). La microscopia detecto 125 (66%) de las muestras con cultivos positivos, menos que las técnicas de cultivo (p<0.0001). La microscopia fue más rápida; los cultivos líquidos positivos fueron detectados en una media de 10 días, más rápido que los 20 días para los cultivos sólidos (p<0.0001). La detección de MDR tuvo una concordancia entre los cultivos solido y líquido de 97%. Un técnico trabajando a tiempo complete puede procesar ~60 microscopias/día, ~30 cultivos sólidos o ~10 cultivos líquidos. El costo más barato fue el de la microscopia, mayor para el cultivo solido y el mucho mayor para el cultivo liquido.

Conclusiones: La microscopia es rápida, barata pero poco sensible y no detecta MDR. El cultivo solido es simple, rápido de realizar y tiene mayor bioseguridad que el cultivo líquido, el cual requiere mayores recursos. El cultivo liquido dar resultados más rápidos pero tiene más fallas; las sensibilidades fueron similares. Este resultado informa de la selección apropiada de la técnicas diagnosticas en diferentes lugares.

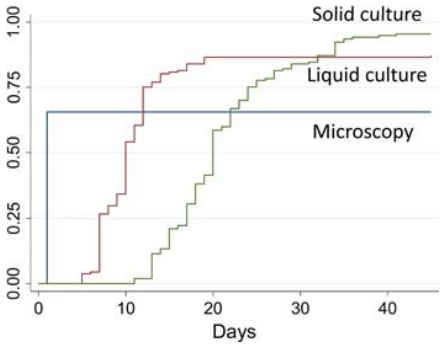


RESUMEN Presentado en la 42 Conferencia Anual de la Unión Internacional contra la Tuberculosis & Enfermedad Pulmonar en Lille, France, Octubre 26 - 30, 2011. Numero de referencia 0001270.

Methods: Adults (n = 544) with suspected TB provided sputum that was split between:

1 unconcentrated Ziehl-Neelsen sputum smear microscopy;
2 solid culture: the MDR/XDRTB Colour Test with disinfection in the sputum pot, the contents of which were applied directly to thin-layer agar that was sealed, incubated and changed colour if microorganisms grew; and
3 liquid culture: the MODS technique that decontaminated sputum with NaOH and centrifugation, re-suspended in broth, incubated and screened microscopically for TB growth.

Results: Fewer solid than liquid-cultures failed due to indeterminate or contaminated results (6/544, 1.1% vs. 77/544, 14%, $P < 0.0001$). Of 168 culture-positive samples with fully interpretable results, 156 (93%) were positive by solid-culture vs. 164 (98%) by liquid-culture ($P = 0.08$). Of 189 culture-positive samples, 173 (92%) were positive in solid-culture vs. 165 (87%) in liquid-culture ($P = 0.2$). Microscopy detected 125 (66%) of the culture-positive samples, less than either culture technique ($P < 0.0001$). Microscopy was most rapid; positive liquid-cultures were detected in median 10 days, faster than 20 days for solid-cultures ($P < 0.0001$). Solid and liquid culture MDR-TB-testing had 97% agreement. A technician working full-time managed ~60 microscopies/day, ~30 solid-cultures or ~10 liquid-cultures. Costs were lowest for microscopy, higher for solid-culture and highest for liquid-culture.



Conclusions: Smear microscopy is rapid, inexpensive but insensitive and cannot test for MDR-TB. Solid culture was simple, quick to perform and had greater biosafety than liquid culture, which required greater resources. Liquid-culture provided results more rapidly but failed more often; sensitivities were similar. These results inform the selection of appropriate diagnostic techniques for different settings.

PC-1270-29 Comparison of TB diagnosis with the MDR-/XDR-TB colour test and MODS

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Background: The diagnosis of microscopy-negative TB and MDR-TB may be achieved by non-commercial solid or liquid-culture techniques that we compared in Peru.

FIELD ASSESSMENT OF TB CULTURE TECHNIQUES IN A RESOURCE-POOR JUNGLE SETTING

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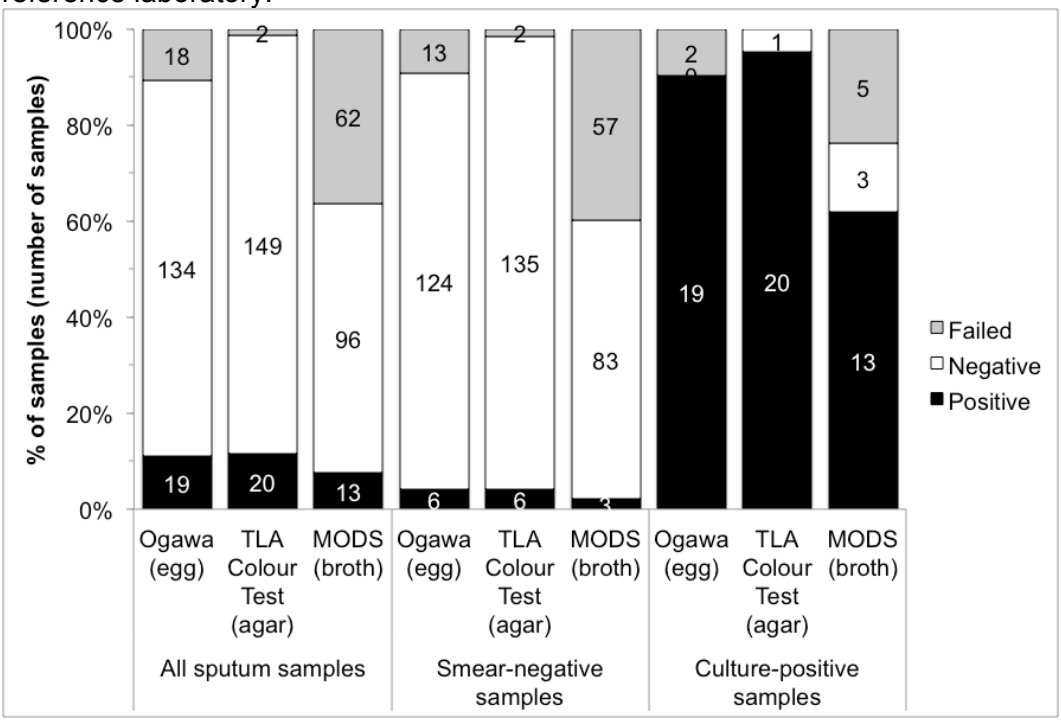
Background: Sputum smear microscopy negative and drug resistant TB principally occur in resource-poor settings but research evaluating tests to diagnose them is usually done under optimized conditions. We therefore assessed the performance of 3 TB culture techniques for samples collected under operational conditions and tested in a basic TB laboratory in the Amazon compared with testing in a distant reference laboratory.
Methods: Sputum samples were collected from patients with suspected pulmonary TB and sent to the local laboratory. Sputum was tested immediately by: direct un-concentrated sputum smear microscopy; modified-Petroff decontamination and Ogawa culture; and the thin-layer agar (TLA) MDR/XDR-TB Colour Test technique. The residual sputum was then refrigerated and flown to a biosafety level-3 reference laboratory for broth culture testing with the MODS (Microscopic-Observation Drug-Susceptibility) technique.
Results: The established Ogawa culture and the newly implemented Colour Test culture had similar ease-of-use and performance (Figure). The broth MODS culture technique had a significantly higher failure rate due to contamination, principally for samples for which un-refrigerated transport from remote jungle regions had delayed culture by more than 2 weeks. Consequently, MODS diagnosed significantly fewer patients than other tests, although it did so significantly more rapidly. The Colour Test and MODS provided concurrent MDR-TB testing results with 100% concordance.
Conclusions: This ongoing study has demonstrated that TB diagnostic test performance was strongly dependent upon delay until testing. For samples that had prolonged unrefrigerated transport, the optimal speed of broth culture was outweighed by frequent contamination. The MDR/XDR-TB Colour Test was successfully implemented in a basic field laboratory under operational conditions and provided significantly greater diagnostic yield than testing in a distant reference laboratory.



Abstract *in press* in the International Journal of TB and Lung Disease and accepted for presentation at the 43rd Union World Conference on Lung Health, to be held in Kuala Lumpur, Malaysia, 13-17 November 2012 www.worldlunghealth.org

INT J TUBERC LUNG DIS 16(8):1113–1118
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http://dx.doi.org/10.5588/ijtld.11.0609

The Colour Test for drug susceptibility testing of *Mycobacterium tuberculosis* strains

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SUMMARY

SETTING: Tartu, Estonia.

OBJECTIVE: To assess the performance and feasibility of the introduction of the thin-layer agar MDR/XDR-TB Colour Test (Colour Test) as a non-commercial method of drug susceptibility testing (DST).

DESIGN: The Colour Test combines the thin-layer agar technique with a simple colour-coded quadrant format, selective medium to reduce contamination and colorimetric indication of bacterial growth to simplify interpretation. DST patterns for isoniazid (INH), rifampicin (RMP) and ciprofloxacin (CFX) were determined using the Colour Test for 201 archived *Mycobacterium tuberculosis* isolates. Susceptibilities were compared to blinded DST results obtained routinely using the BACTEC™ Mycobacteria Growth Indicator Tube™ (MGIT) 960 to assess performance characteristics.

RESULTS: In all, 98% of the isolates produced interpretable results. The average time to positivity was 13 days, and all results were interpretable. The Colour Test detected drug resistance with 98% sensitivity for INH, RMP and CFX and 99% for multidrug-resistant tuberculosis. Specificities were respectively 100% (95%CI 82–100), 88% (95%CI 69–97) and 91% (95%CI 83–96) and 90% (95%CI 74–98). Agreement between the Colour Test and BACTEC MGIT 960 were respectively 98%, 96%, 94% and 97%.

CONCLUSION: The Colour Test could be an economical, accurate and simple technique for testing tuberculosis strains for drug resistance. As it requires little specialist equipment, it may be particularly useful in resource-constrained settings with growing drug resistance rates.

KEY WORDS: Estonia; fluoroquinolones; multidrug-resistant tuberculosis; thin-layer agar

APPROXIMATELY 0.5 million cases of multidrug-resistant tuberculosis (MDR-TB) are reported each year, and extensively drug-resistant TB (XDR-TB) has been recorded in a substantial number of countries worldwide.[1] Despite a reported small decrease in TB incidence in some countries, the overall proportion of MDR-TB disease is rising, and many of the settings with high rates of MDR-TB have limited resources in terms of detection of TB and drug-resistant disease.

There is a global need for new reliable and affordable methods of the detection of drug resistance, as rapid identification of MDR- and XDR-TB is vital for the prompt initiation of adequate treatment and interruption of further transmission of resistant strains.[2,3] Three main drug susceptibility testing (DST) strategies are currently in routine use: on solid culture, on liquid culture and molecular methods. Traditional solid culture-based DST, which is less costly than liquid-based methods, can take weeks to yield reliable results. The BACTEC™ Mycobacteria Growth Indicator Tube™ 960 (MGIT; BD, Sparks, MD, USA) liquid culture is a rapid and highly sensitive DST method.[4–9] Although the MGIT system is currently considered the gold standard in DST, it nevertheless has the following disadvantages: 1) the requirement for dense liquid cultures for diagnosis; 2) difficulty in obtaining specialist reagents, particularly in smaller low-income countries; and 3) high costs, which prohibit its use in low-income countries. Molecular tests also require expensive equipment,[10–15] and most also require specialised expertise. New methods are therefore needed for low-income countries with a high incidence of TB and a growing problem of drug resistance.

Mycobacterium tuberculosis microcolony detection on thin-layer agar (TLA) has been in use for a long time.[16–18] It has been evaluated on primary specimens against other culture methods such as MGIT and Löwenstein-Jensen (LJ) media, with excellent results.[19–23] TLA is an inexpensive non-commercial technique, requiring only a 37°C incubator and a light microscope to confirm diagnosis. The TLA

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Article submitted 2 September 2011. Final version accepted 6 March 2012.

MDR/XDR-TB Colour Test has recently been developed in collaboration with the Foundation of Innovative New Diagnostics for the identification of *M. tuberculosis* complex and detection of resistance to isoniazid (INH), rifampicin (RMP) and ciprofloxacin (CFX).[24] Although the use of CFX is not currently recommended for the treatment of MDR-TB, several studies have shown a high degree of cross-resistance between fluoroquinolones (FQs).[25,26]

The Colour Test in its current version tests susceptibility to CFX as a proxy for the identification of FQ resistance. The test is based on the TLA method, with the addition of an oxidation-reduction indicator: 2, 3 diphenyl-5-(2-thienyl) tetrazolium chloride (STC),[27,28] and colour-coded quadrants to simplify the reading of the DST results. The addition of STC to the media results in the growth of red TB colonies and thus makes them visible to the naked eye at early stages of growth. This makes daily checking of the plates faster, as there is no need to check every plate under the microscope. A microscope is still needed, however, to confirm a positive diagnosis of *M. tuberculosis* based on colony morphology. The Colour Test was extensively tested for the rapid diagnosis of TB directly from sputum samples.[29] However, no data are available on its performance on cultures, although this might be necessary should the test be applied at a reference level using isolates grown from different types of specimens.

The present study evaluates the performance characteristics and feasibility of the Colour Test in the identification of TB from cultures, concurrently with the detection of MDR-TB strains and resistance to CFX, against a gold standard of conventional phenotypic DST using MGIT.

MATERIALS AND METHODS

Study material

Routine strains archived during the years 2004–2010 were selected sequentially from the most recently archived isolates to older ones at the National TB Reference Laboratory, Tartu University Hospital, Tartu, Estonia. All of the isolates included had been previously identified as *M. tuberculosis* by AccuProbe (GenProbe Inc, San Diego, CA, USA) or GenoType® MTBC (Hain Lifescience, Nehren, Germany), and had RMP and INH DST results available that had been obtained using MGIT. All MDR-TB strains were tested using MGIT for susceptibility to second-line drugs. Twenty of the selected non-MDR-TB strains did not have second-line DST results.

Of the 201 isolates used in the study, 30 were non-MDR-TB and 171 were MDR-TB strains: 82 of the MDR-TB isolates were also resistant to ofloxacin (OFX), and 40 of these were XDR-TB strains, i.e., further resistant to at least one of the injectable drugs (amikacin, kanamycin or capreomycin).

Staff participating in the study were blinded to the original phenotypical DST results. Blinding was performed by an independent staff member not involved in the testing.

Introduction of the test

Before the start of the study, the Colour Test method was standardised using 10 strains from routine laboratory work with known first- and second-line DST results. The best fitting dilutions of the inoculum were determined using several dilutions with sterile water of McFarland 1 standard: 1/10, 1/100, 1/1000 and 1/10 000. It was found that McFarland 1 standard dilutions at 1/10, and sometimes 1/100, had the optimal colony count (50–500 colonies in the control quadrant); consequently, both these dilutions were used in this study to plate the 201 isolates on Colour Test plates.

A new investigator from the field site (National TB Reference Laboratory, Estonia) underwent a 3-day training course in the Colour Test technique at the UK Health Protection Agency National Mycobacterium Reference Laboratory prior to the study. Post-training photos of plates were taken and sent electronically to London and Peru, and the plates were re-read to guide and monitor the success of initial training and to monitor and correct errors.

Panel preparation

Strains were subcultured on LJ media using 250 µl of thawed isolate. At the same time, the initial cryovial was checked for contamination by plating on blood agar and incubating at 37°C for 48 h. The presence of acid-fast bacilli on positive LJ slopes was confirmed by Ziehl-Neelsen staining.[30]

Drug susceptibility testing

First- and second-line DST was performed using the automated liquid MGIT system according to the manufacturer's guidelines and World Health Organization recommended drug concentrations: 0.1 µg/ml for INH, 1.0 µg/ml for RMP[31] and 2.0 µg/ml for OFX.[32] DST was performed at the National TB Reference Laboratory, Tartu University Hospital.

Colour Test method

Middlebrook 7H11 agar was used to prepare the Colour Test plates. The medium was supplemented with oleic acid albumin dextrose complex (OADC) supplement (10%), Mycobacteria Selectatab (Kirchner, Mast Laboratories Ltd, Merseyside, UK) and 50 µg/ml of STC (TCI Europe, Zwijndrecht, Belgium). Quadrant petri dishes were prepared with approximately 4 ml of agar per quadrant, one with 0.2 µl/ml INH and green food colouring, one with 1 µl/ml RMP and yellow food colouring, and one with 2 µl/ml CFX and blue food colouring. Food colouring was supplied by Dr Oetker (Leeds, UK). All food colouring was filter-sterilised using a 0.22 µm filter (Millipore, Billerica, MA, USA). The remaining quadrant contained no drug

concentration and acted as the control for growth detection. The plates were screened for contamination every time the medium was prepared. Internal quality control was performed with a susceptible (H37Ra) and a resistant strain (laboratory origin).

Strain inocula for plating were prepared in a 50 ml centrifuge tube containing two drops of sterile distilled water and approximately 10 2-mm glass beads. Multiple *M. tuberculosis* colonies grown on LJ medium were added to the water. The tube was vortexed for 30 s and allowed to stand for 20 min. The inoculum was diluted and plated on Colour Test plates using two drops per quadrant. The plates were sealed with parafilm (American National Can Company, Norwalk, CT, USA) and placed in a sealed zip-lock plastic bag for safety reasons. Plates were incubated at 37°C in room air.

The plates were read visually every other day until at least 50 colonies appeared in the drug-free control quadrant. Growth was detected as red colonies due to a redox reaction with STC. The presence of *M. tuberculosis* was confirmed using a conventional light microscope (magnification 50×). A strain was classed as resistant to a drug when >1% of colonies appeared in a drug quadrant compared to the control quadrant. Colour Test plates were also re-read 21 days after inoculation.

Statistical power and sampling

The number of selected isolates with different DST patterns was based on power calculations that assumed comparable sensitivity and specificity for standard phenotypic testing for all tested drugs based on the published data of DST testing on TLA plates directly with specimens, i.e., not less than 85% for both sensitivity and specificity.

Statistical analysis was performed using Microsoft Excel (Microsoft, Redwoods, WA, USA) and STATA (StataCorp LP, College Station, TX, USA). The main outcome measures were the performance characteristics of the Colour Test assay: total agreement between experimental and the reference tests, sensitivity, specificity, likelihood ratios for a negative and a positive result (LR− and LR+) and the cost of a Colour Test plate in Estonia.

Ethics statement

As no patient information was used in this study, ethics approval was not sought.

RESULTS

The retail cost for materials for one Colour Test plate using local suppliers for all the reagents was 2.4€ (US$3.1). The resistant and susceptible control strains always yielded correct results, and none of the Colour Test plates were contaminated. Standardisation of the Colour Test method was required to exclude unnecessary dilutions that did not yield enough colonies in the control quadrant or were too dense. No uncertain results in interpreting DST from the Colour Test plates were therefore obtained during this study.

Of the 201 selected isolates tested on the Colour Test plates, 98% gave interpretable results. Four strains showed insufficient growth (<50 colonies in the control quadrant) or no growth on the Colour Test plates despite several attempts. The results from 197 strains were thus used for analysis. To evaluate CFX susceptibility, strains with available MGIT DST results for second-line drugs were used (n = 177). The mean time to detection was 13 days (range 6–21), and all results were interpretable.

The Table demonstrates test performance parameters. The total agreement for individual drugs between

Table Performance characteristics of the thin-layer agar MDR/XDR-TB Colour Test (n = 197 strains)

	MGIT susceptible	DST resistant	Sensitivity % (95%CI)	Specificity % (95%CI)	Total agreement % (95%CI)	LR+ (95%CI)	LR− (95%CI)
INH Colour Test							
Susceptible	19	3	98 (95–100)	100 (82–100)	98 (96–100)	0	0.02 (0.01–0.05)
Resistant	0	175					
RMP Colour Test							
Susceptible	22	4	98 (94–99)	88 (69–97)	96 (93–99)	8.14 (2.81–23.5)	0.03 (0.01–0.07)
Resistant	3	168					
CFX Colour Test							
Susceptible	89	2	98 (91–100)	91 (83–96)	94 (89–97)	10.6 (5.69–19.8)	0.03 (0.01–0.11)
Resistant	9	77					
MDR-TB Colour Test							
Susceptible	27	2	99 (96–100)	90 (74–98)	97 (94–99)	9.88 (3.38–28.9)	0.01 (0–0.05)
Resistant	3	165					
MDR-TB+CFX− Colour Test							
Susceptible	89	2	97 (91–100)	90 (82–95)	93 (88–96)	9.65 (5.35–17.4)	0.03 (0–0.11)
Resistant	10	76					

MGIT = Mycobacteria Growth Indicator Tube; DST = drug susceptibility testing; CI = confidence interval; LR+ = likelihood ratio for a positive result (indicates how much more likely a strain with resistance to a given drug will have a positive [i.e., resistant] result on the Colour Test plate than a strain susceptible to that given drug); LR− = likelihood ratio for a negative result (indicates how much more likely a strain susceptible to a given drug has a negative [i.e., susceptible] result on the Colour Test plate, compared to a strain that is resistant to that given drug); INH = isoniazid; Colour Test = thin-layer agar MDR/XDR-TB Colour Test); RMP = rifampicin; CFX = ciprofloxacin; MDR-TB = multidrug-resistant tuberculosis; MDR+CFX− = multidrug- and ciprofloxacin-resistant.

the MGIT and the Colour Test varied between 96% (95% confidence interval [CI] 93–99) and 98% (95%CI 96–100) for RMP and INH, respectively, and 94% (95%CI 89–97) for CFX. Overall agreement for detection of MDR-TB was 97% (95%CI 94–99), while it was lower (93%, 95%CI 88–96) for the detection of resistance to all three drugs simultaneously. Test sensitivity was 97% (95%CI 91–100) for detecting resistance to all three drugs at the same time; 98% for detecting resistance to RMP (95%CI 94–99), INH (95%CI 95–100) or CFX (95%CI 91–100); and 99% (95%CI 96–100) for detecting MDR-TB. Specificities varied between 88% (95%CI 69–97) for RMP and 100% (95%CI 82–100) for INH. Specificity for FQs was 91% (95%CI 83–96). The Figure shows the Colour Test colony counts for each of the drugs, demonstrating that there were few borderline results.

Colour Test re-readings on day 21 had no significant effect on INH or RMP results; however, late growth in the CFX quadrant caused a significant decrease in DST agreement with MGIT ($P = 0.03$, data not shown).

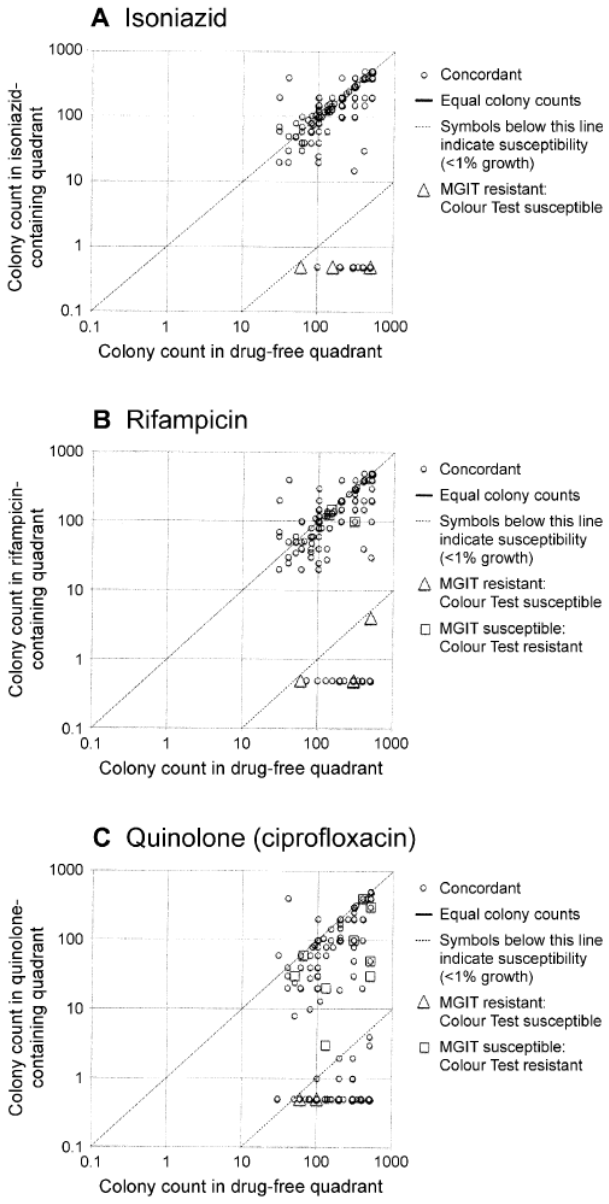

Figure Colour Test colony count results. The graphs show the number of *M. tuberculosis* colonies visible to the naked eye on the day that each test became culture-positive. Drug resistance results that were concordant and discordant with the MGIT test are indicated by different symbols (see legend). Strains were considered susceptible to a drug if it prevented more than 99% of tuberculosis growth (dotted line). MGIT = Mycobacteria Growth Indicator Tube.

DISCUSSION

This is the only study so far to pragmatically evaluate the performance of the Colour Test for the detection of resistance to INH, RMP and CFX on clinical isolates as an alternative to commercial testing methods. This inexpensive, non-commercial test allows for the diagnosis of MDR-TB; moreover, it can serve as an exclusion test for XDR-TB by analysing resistance to CFX.

Our study demonstrated good performance of the Colour Test. The test proved inexpensive; however, appropriately designed cost-effectiveness studies are needed to assess this formally. The cost of retail materials needed to produce one Colour Test plate was 2.4€ when all reagents were purchased in Estonia; this is similar to estimates published in 2009 by Martin et al. for TLA.[20] Although, the Colour Test DST performance time was somewhat slower than the MGIT DST at 13 days, it was significantly faster than other solid-media DST.[6]

In a routine diagnostic laboratory, it was feasible to introduce the Colour Test method within a relatively short period of time. The addition of Selectatab to the Colour Test media did not inhibit the growth of TB, and none of the plates were contaminated throughout the study. The addition of STC to the media made the red colonies visible to the naked eye in early stages of growth, and the addition of food colouring to the detection quadrants facilitated rapid preparation and easier reading of the plates. The prior standardisation of the dilutions used allowed for easy interpretation of the DST results.

The high total agreement between MGIT DST and excellent LR− and LR+ values suggests that this technique can serve as an accurate and promising test. The FQ DST agreement between the Colour Test and MGIT was highest when the Colour Test was read on the day that TB growth was first detected; interpretation should therefore not be delayed after this time. The test shows excellent sensitivity for all the drugs tested, and the specificity was excellent for INH, while the specificity for RMP and CFX was lower than expected. The MGIT DST results were obtained prior to cryopreservation, whereas the Colour Test results were obtained after cryopreservation and subculture of the TB strain. This methodological issue may

have contributed to the discrepancies observed, and future research should ideally compare different tests performed concurrently on the same passage of a strain. The lower specificity for RMP and a wide CI might result from a very low proportion of susceptible strains in the panel (only 25/197 strains were RMP-susceptible).

One of the study limitations was the indirect comparison of the test performance in detecting sensitivity to FQs. Possible absence of complete cross-resistance between CFX and OFX might have resulted in the lower specificity for this group of drugs. However, evidence of cross-resistance has been reported elsewhere;[25,26] the results presented are therefore somewhat controversial. Due to technical and financial constraints, no sequencing of the tested isolates was conducted. Further modifications to the Colour Test methodology permitting further testing for OFX or moxifloxacin, as performed by Martin et al. in 2009, are needed.[33]

Overall, the results of this study demonstrate similar good performance characteristics when used indirectly on isolates compared to previous studies with its progenitor, the TLA test, on direct specimens.[22,23] The advantages of the indirect method are possible application using cultures grown from all types of pulmonary and extra-pulmonary biological specimens, and as a replacement or back-up for commercial methods. Another advantage of this method is that it is relatively safe in environments where the relative risk of infection with *M. tuberculosis* cultures is high.[34] For the Colour Test technique, isolates are plated in a biological safety cabinet, and the plate is sealed and bagged securely; consequently, if the plate is dropped, no culture can escape.

This study suggests that, with appropriate training and careful attention to methodology, the Colour Test is a reliable technique. It is relatively rapid, with high accuracy for MDR-TB detection, and is suitable for low-income countries as a rapid and more economical alternative to liquid culture systems. Direct testing on different types of primary specimens could be of even higher diagnostic value to MDR-TB suspects. The Colour Test should therefore be further evaluated in high-resistance settings using direct specimens, as this could further illustrate the advantages of this safe and economical test.

Acknowledgements

The research leading to these results received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement FP7-223681. CAE was supported by IFHAD and the Wellcome Trust. The funders had no role in the design or analysis of the study.

THE SPECIFICITY OF MDR/XDR-TB COLOUR TEST FOR DIFFERENTIATING MYCOBACTERIUM TUBERCULOSIS FROM ATYPICAL MYCOBACTERIA

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BACKGROUND: For culture-based TB diagnosis in resource-limited settings, M. tuberculosis is usually differentiated from atypical mycobacteria by the presence of cording morphology. However, the reliability of this approach is poorly characterised and has not been reported for the new MDR/XDR-TB Colour Test technique in which samples are decontaminated in the sputum pot and applied directly to a sealed thin-layer agar culture plate that changes colour if TB grows, providing concurrent MDR-TB testing and XDR-TB screening in basic laboratories.

METHODS: Positive mycobacterial cultures (n=4,371) from the Microscopic-Observation Drug-Susceptibility (MODS), Ogawa, Lowenstein Jensen (LJ) and Colour Test culture techniques were retested with the gold standard immunochromatographic Capilia TB Neo rapid assay for *M. tuberculosis* complex that was performed according to the manufacturers' instructions using 80 µl from MODS broth cultures or colonies from solid cultures suspended in extraction buffer. All tests were performed blinded to all other study data and proportions are stated with their 95% confidence intervals (95%CI).

RESULTS: Examination of culture colonies revealed cording morphology in 4362/4405 (99.02%). The proportion of these cultures that were morphologically mis-identified as M. tuberculosis but were defined by the Capilia test to be atypical mycobacteria was 5/1514 (0.33%, 95% CI 0.11-0.77%) for MODS, 1/1044 (0.096%, 95% CI 0.024-0.53%) for Ogawa, 0/848 for LJ and 1/955 (0.10% 95%CI 0.0027-0.58%) for the Colour Test (P>0.1), indicating >99.6% specificity. Atypical mycobacteria were uncommon and all 43/43 (100%) of cultures with non-cording morphology were confirmed by the Capilia test to be atypical mycobacteria, indicating 100% (95% CI 92-100%) specificity.

CONCLUSION: The morphological differentiation of M. tuberculosis from atypical mycobacteria was highly reliable for MODS, LJ, Ogawa and the thin-layer agar MDR/XDR-TB Colour Test culture techniques.

THE MDR/XDR-TB COLOUR TEST SIMULTANEOUS CULTURE AND DRUG SUSCEPTIBILITY RESULTS PREDICT TUBERCULOSIS OUTCOMES

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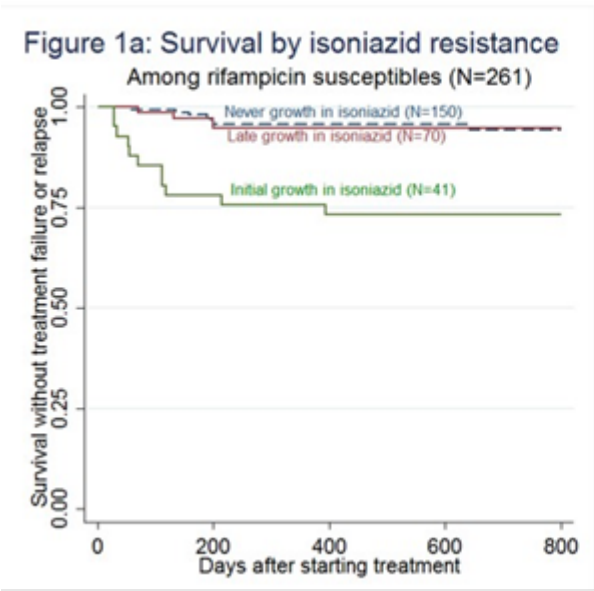
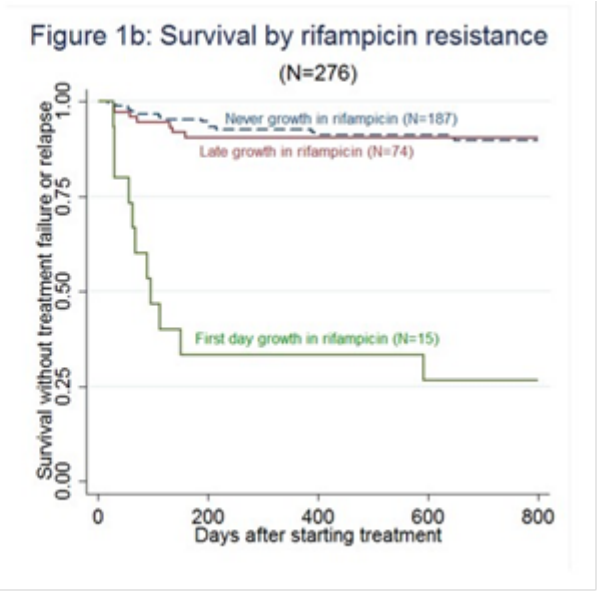
Background: Commitment to treating multi-drug resistant (MDR) TB is growing. Many peripheral laboratories lack the facilities to perform drug susceptibility testing (DST), relying on indirect DST performed in reference labs, often with delays of several months. The "MDR/XDR-TB Colour Test" is a thin layer agar culture method with improved biosafety designed for use in basic laboratories that allows simultaneous TB culture and DST for isoniazid, (H), rifampin, (R), and ciprofloxacin.

Design/Methods: DST results for the Colour Test are assigned by growth in drug-containing agar on the first day of TB growth. On occasion there is delayed growth in a drug-containing well in a plate initially read as susceptible. We followed up 503 episodes of TB in 492 patients diagnosed between April, 2009 and February, 2011 and used clinical outcomes to assess the clinical significance of different TB growth patterns in the Colour Test. Desirable outcomes were defined as cure or finished treatment. Adverse outcomes were TB recurrence, all-cause mortality, and treatment failure. Hazard ratios (HR) were obtained with Cox regression for H and R separately. We analyzed H-resistance in R-susceptible strains only.

Results: 43 adverse patient outcomes occurred during 521 patient-years of follow-up. Colour Test DST results were available for 79% (399) of the samples. The median time from sample inoculation to results was 20 days (IQR: 17-22; maximum 45 days). Among the 351 R-susceptible strains, 16% (57) were H mono-resistant and another 28% (97) had delayed growth in H. Overall 11% (43) were MDR strains and 1.3% (5) were R mono-resistant. Another 26% (103) had late growth in R. Figures 1a and 1b show survival curves for H and R respectively. Initial growth in H had an HR of 6.0 (95%CI: 2.2, 17; p=0.001) for adverse outcomes compared to never growth in H. Late growth was similar to never growth (HR: 1.0; 95%CI: 0.3, 2.1; p=1.0). Early growth in R predicted adverse outcomes with HR=17 (95%CI: 7.4, 40; p<0.001). Late growth was not significantly associated with outcomes (HR 1.5, 95%CI:0. 6, 3.9; p=0.9) compared to never growth in R.

Conclusions: The Colour Test provides clinically meaningful drug susceptibility results at the time of first TB growth in an average of 3 weeks and maximum 6 weeks. At this time positive cultures can immediately have a final report sent and the cultures can be discarded or sent for further analysis without further incubation.





EL CULTIVO SIMULTÁNEO PRUEBA DE COLORES TB-MDR/XDR Y LOS RESULTADOS DE SENSIBILIDAD A DROGAS PREDICEN LOS RESULTADOS DE TUBERCULOSIS.

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Background: El compromiso con el tratamiento de TB multidrogoresistente (TB-MDR) es cada vez mayor. Muchos laboratorios periféricos carecen de las instalaciones necesarias para llevar a cabo pruebas de sensibilidad a drogas, basándose en pruebas de sensibilidad indirecta realizada en los laboratorios de referencia, a menudo con retrasos de varios meses. La "Prueba de Colores TB-MDR/XDR" es un método de cultivo de agar de capa delgada con una mejor bioseguridad diseñada para su uso en laboratorios básicos que permite cultivar en forma simultánea TB y pruebas de sensilbilidad para isoniazida (H), rifampicina (R) y ciprofloxacina

Diseño/Métodos: Los resultados de la prueba de sensibilidad a drogas en la Prueba de Colores son asignados mediante el crecimiento en el agar que contiene drogas en el primer día de crecimiento de TB. De vez en cuando hay una demora en el crecimiento en el cuadrante que contiene una droga leído inicialmente como sensible. Se dio seguimiento a 503 episodios de TB en 492 pacientes diagnosticados entre abril de 2009 y febrero de 2011 y se utilizó los resultados clínicos para evaluar la significación clínica de los diferentes patrones de crecimiento de la tuberculosis en la Prueba de Colores. Resultados deseables se definieron como cura o con tratamiento finalizado. Resultados adversos fueron recurrencia de TB, todas las causas de mortalidad y el fracaso del tratamiento. Los

coeficientes de riesgos (HR) se obtuvieron con la regresión de Cox para H y R por separado. Se analizaron resistencia a H sólo en cepas susceptibles a R.

Resultados: 43 resultados adversos de pacientes ocurrieron en 521 pacientes en años de seguimiento. Los resultados de la prueba de sensibilidad en la Prueba de Colores fueron disponibles para el 79% (399) de las muestras. El tiempo promedio desde la inoculación de la muestra hasta la obtención de los resultados fue de 20 días (IQR: 17-22; máximo 45 días). Entre las 351 cepas susceptibles a R, 16 % (57) eran monoresistente a H y otro 28 % (97) había retraso en el crecimiento en H. En general 11 % (43) eran cepas resistentes a múltiples fármacos y 1,3 % (5) fueron de monoresistente a R. Otro 26 % (103) tuvo un crecimiento tardío en R. Figuras 1a y 1b muestran las curvas de supervivencia de H y R, respectivamente. El crecimiento inicial en H tuvo un HR de 6,0 (IC del 95 %: 2,2 , 17 , p = 0,001) para los resultados adversos en comparación con los que nunca tuvieron crecimiento en H. El crecimiento tardío fue similar en aquellos sin crecimiento (HR: 1,0, IC 95 %: 0,3; 2,1, p = 1,0). El crecimiento inicial en R predijo resultados adversos con HR = 17 (IC del 95 %: 7,4 , 40 , p < 0,001). El crecimiento tardío no se asoció significativamente con los resultados (HR 1,5 , IC 95 %: 0 6 , 3,9 , p = 0,9) en comparación con aquellos sin crecimiento en R.

Conclusiones: La Prueba de Colores proporciona resultados clínicamente significativos a la sensibilidad a los medicamentos al momento del primer crecimiento de TB en un promedio de 3 semanas y un máximo de 6 semanas. En este momento los cultivos positivos pueden tener de inmediato un informe final enviado y los cultivos pueden ser descartados o enviados para su análisis sin necesidad de incubación.

Reference of your abstract	OP-242-02
Title of abstract	The MDR/XDR-TB Colour Test simultaneous culture and drug susceptibility results predict tuberculosis outcomes
Type of session	Oral Abstract session
Title of session	18. Advances in culture methods
Date and time	Saturday, 2 November 2013 from 12:45 to 14:15
Room	Announced in the Final Programme of the conference

(This session will be co-chaired by Carlton Evans)

CARBENDAZIM REDUCES CONTAMINATION OF TB CULTURES IN THE THIN LAYER AGAR MDR/XDR-TB COLOUR TEST

ES Ramos[1], D Coleman[1,3], CE Osorio[1], TR Valencia[1], MF Llacza[1], MA Tovar[1,2], T Wingfield[1,2,4], CA Evans[1,2,4]

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Background: Culture contamination challenges TB diagnosis by confusing, delaying or preventing *M. tuberculosis* detection. Resultant diagnostic delay harms patient outcomes. Despite having lower rates of contamination than broth culture, contamination remains a problem in thin layer agar (TLA) TB diagnosis using the MDR/XDR-TB Colour Test technique. We aimed to reduce contamination using the broad-spectrum benzimidazole fungicide, carbendazim.

Methods: Sputum specimens were collected from people with suspected TB disease who lived in the study site of Ventanilla, on the outskirts of northern Lima, Peru. Between January 2012 and April 2013, 1,736 sputum specimens were cultured using TLA after sputum samples were decontaminated by mixing with trisodium phosphate in the sputum pot. The TLA plates were divided into four separate, non-communicating quadrants, two of which contained carbendazim 50mg/l. The other two quadrants contained no carbendazim. The TLA plates were assessed over 6 weeks for signs of TB growth or contamination in any of the four quadrants. Contamination was defined as bacterial, fungal or mixed on the basis of the recognized morphology of each.

Results: The TLA culture quadrants containing carbendazim were overgrown by contamination in 4/3472 (0.12%), less than 38/3472 (1.1%) of the TLA quadrants that did not contain carbendizim ($P<0.001$). The TLA culture half-plates containing carbendazim were overgrown by contamination in 1/1736 (0.058%), less than 10/1736 (0.58%) of the TLA half-plates that did not contain carbendizim ($P<0.001$). One of the 1736 TLA plates (0.058%) was completely overgrown by fungus in all four quadrants with and without carbendizim whereas all the other 1735/1736 (99.9%) of TLA culture plates gave interpretable results in at least one quadrant. Only fungal contamination was found, there was no evidence of bacterial or mixed infection. The proportion of quadrants with growth of *Mycobacterium tuberculosis* and/or of partial fungal contamination that did not inhibit culture interpretation was unaffected by carbendazim.

Conclusions: The morphological differentiation of *M. tuberculosis* from fungal contamination was highly achievable in TLA TB diagnosis. Carbendazim almost completely prevented fungal contamination in TLA plates causing uninterpretable culture results. Carbendazim is a useful addition to TLA plates to prevent contamination of samples during TB diagnosis and thus increase diagnostic yield.

Quadrants P=0.0001		With carbendazim	
		Totally contaminated	Interpretable
Without carbendazim	Totally contaminated	3	35
	Interpretable	1	3472
Half culture plates P=0.0001		With carbendazim	
		Totally contaminated	Interpretable
Without carbendazim	Totally contaminated	1	9
	Interpretable	0	1736

EL CARBENDAZIM REDUCE LA CONTAMINACIÓN DE LOS CULTIVOS DE TB EN AGAR DE CAPA DELGADA EN LA PRUEBA DE COLORES TB-MDR/XDR

ES Ramos[1], D Coleman[1,3], CE Osorio[1], TR Valencia[1], MF Llacza[1], MA Tovar[1,2], T Wingfield[1,2,4], CA Evans[1,2,4]

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Antecedentes: La contaminación de los cultivos desafía al diagnóstico de TB al confundir, retrasar o prevenir la detección de *M. tuberculosis*. La demora en el diagnóstico resulta perjudicial a los resultados del paciente. A pesar de tener índices más bajos de contaminación que en cultivo líquido, la contaminación sigue siendo un problema en cultivos de agar de capa delgada (TLA) en el diagnóstico de TB utilizando la técnica Prueba de Colores TB-MDR/XDR. El objetivo fue reducir la contaminación mediante el carbendazim, fungicida benzimidazol de amplio espectro.

Métodos: Se colectaron muestras de esputo de personas con sospecha de enfermedad de TB que vivían en el área de estudio de Ventanilla, en las afueras del norte de Lima, Perú. Entre enero de 2012 y abril de 2013, 1736 muestras de esputo fueron cultivadas usando TLA después que las muestras de esputo fueran decontaminadas mediante la mezcla con el fosfato trisódico en el frasco del esputo. Las placas TLA se dividieron en cuatro separados y no comunicantes cuadrantes, dos de los cuales contenía carbendazim 50 mg/l. Los otros dos cuadrantes no contenían carbendazim. Las placas TLA se evaluaron a las 6 semanas mediante el crecimiento de TB o contaminación en cualquiera de los cuatro cuadrantes. La contaminación se define como bacteriana, fúngica o mixta sobre la base de la morfología de cada una

Resultados: Los cuadrantes del cultivo TLA que contienen carbendazim estaban cubiertas por la contaminación en 4/3472 (0,12 %), menos que 38/3472 (1,1 %) de los cuadrantes TLA que no contenían carbendazim (P<0,001). La mitad de la placa de los cultivos TLA que contienen carbendazim estaba cubierta por la contaminación en 1/1736 (0,058 %), menos que 10/1736 (0,58 %) de la mitad de la placa TLA que no contenía carbendazim (P < 0,001). Una de las placas de TLA 1736 (0,058 %) fue completamente cubierta por hongos en los cuatro cuadrantes con y sin carbendazim mientras que todos los demás 1735/1736 (99,9 %) de placas de cultivo de TLA dió resultados interpretables en al menos un cuadrante. Sólo se encontró contaminación por hongos, no

hubo evidencia de contaminación bacteriana o mixta. La proporción de cuadrantes con el crecimiento de *Mycobacterium tuberculosis* y/o de la contaminación parcial fúngica que no inhibió la interpretación del cultivo no fue afectado por el carbendazim.
Conclusiones: La diferenciación morfológica de *M. tuberculosis* de la contaminación por hongos fue muy alcanzable en el diagnóstico de TB en TLA. El carbendazim casi completamente impidió la contaminación fúngica en placas TLA causante de resultados no interpretables en el cultivo. El carbendazim es un suplemento útil a las placas de TLA para evitar la contaminación de las muestras durante el diagnóstico de la tuberculosis y por lo tanto aumentar el rendimiento del diagnóstico.

Reference of your abstract	OP-243-02
Title of abstract	Carbendazim reduces contamination of TB cultures in the thin layer agar MDR/XDR-TB Colour Test
Type of session	Oral Abstract session
Title of session	18. Advances in culture methods
Date and time	Saturday, 2 November 2013 from 12:45 to 14:15
Room	Announced in the Final Programme of the conference

(This session will be co-chaired by Carlton Evans)

COMPARISON OF TIME TO MDR-TB DIAGNOSIS WITH THE MDR/XDR-TB COLOUR TEST & CURRENT ALGORITHM IN A RESOURCE-POOR JUNGLE SETTING IN PERU

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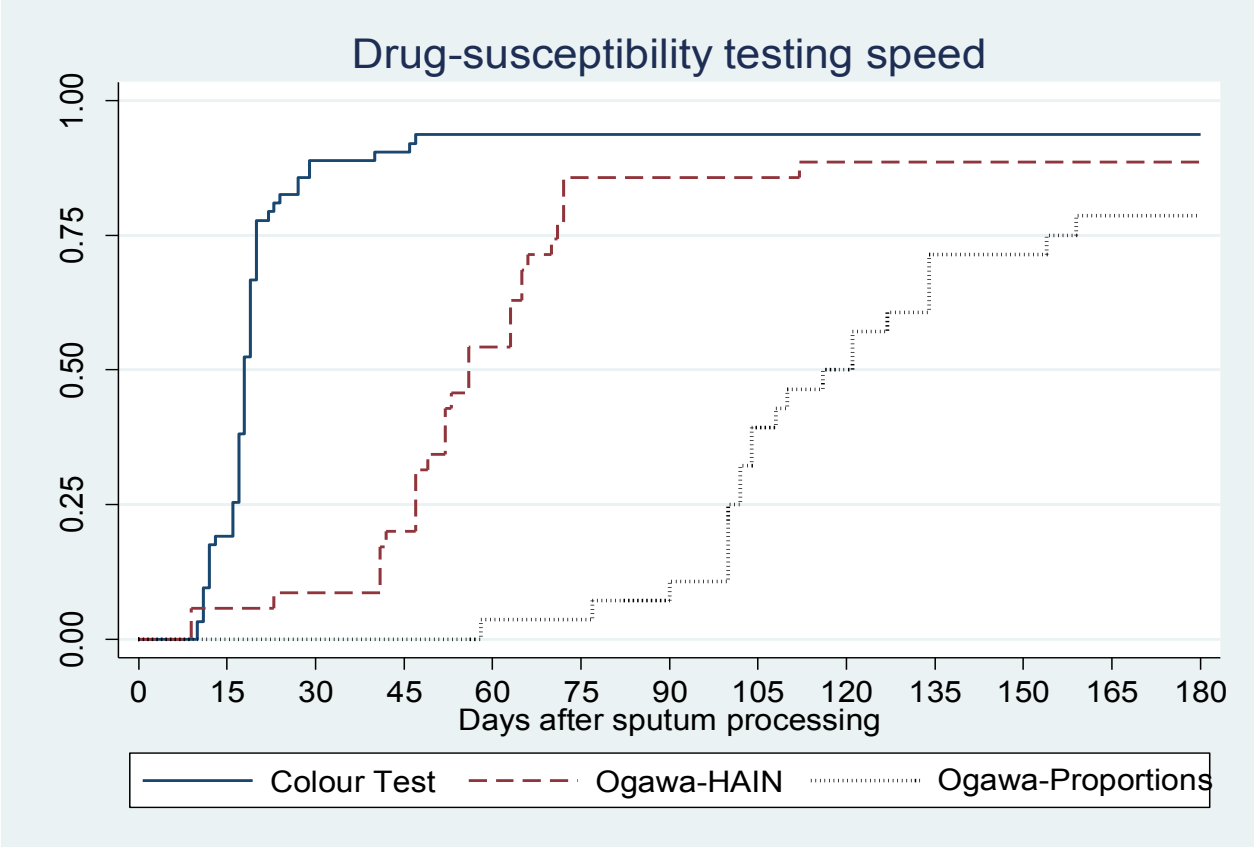
Background: The diagnosis of MDR-TB in resource-poor settings is challenging due to limited capacity for local testing and delays involved in shipping samples to distant reference laboratories. We therefore evaluated the MDR/XDR-TB Colour Test, a thin-layer agar technique in which sputum pot contents are applied directly to a colorimetric culture plate that is then permanently sealed and provides concurrent TB detection and drug-susceptibility testing

Methods: Sputum samples (n=697) were collected from patients with suspected pulmonary TB and sent to the local laboratory in the Peruvian Amazon. This was equipped with a biosafety cabinet, incubator and microscope but no other TB-related equipment. Sputum was tested immediately by the current standard algorithm using modified-Petroff decontamination and Ogawa culture (without centrifugation). Ogawa positive cultures were then shipped by air to the national reference laboratory for drug-susceptibility testing using (1) rapid molecular testing with the Hain test and/or (2) traditional culture-based testing with the proportions technique. Additionally for this research project, residual sputum was (3) added to disinfectant and immediately applied to the MDR/XDR-TB Colour Test, permanently sealed and incubated locally. All cultures were read weekly. Time to drug-susceptibility testing results were compared between the three diagnostic approaches.

Results: Drug-susceptibility testing results were attempted for 63 samples that were culture-positive by any test. 87% (55/63) were Ogawa culture-positive and all had drug-susceptibility testing results available from the national laboratory. Time from sputum processing to results was median 54 days for Ogawa culture followed by Hain testing and 112 days with the proportions test. Local direct drug-susceptibility testing with the MDR/XDR-TB Colour Test provided results for 94% (59/63) samples and time from sputum processing to drug-susceptibility testing results was median 19 days, significantly faster than the other tests (Figure).

Conclusions: Local drug-susceptibility testing directly from sputum with the MDR/XDR-TB Colour Test was successfully implemented in a basic jungle laboratory and provided results considerably more rapidly than the current diagnostic algorithm that uses distant testing of positive cultures with a rapid molecular technique.



COMPARACIÓN DEL TIEMPO PARA DIAGNOSTICAR TB-MDR ENTRE LA PRUEBA DE COLORES PARA TB MDR/XDR & EL ALGORITMO DE DIAGNÓSTICO ACTUAL EN CONTEXTOS DE RECURSOS LIMITADOS DE LA SELVA DEL PERÚ

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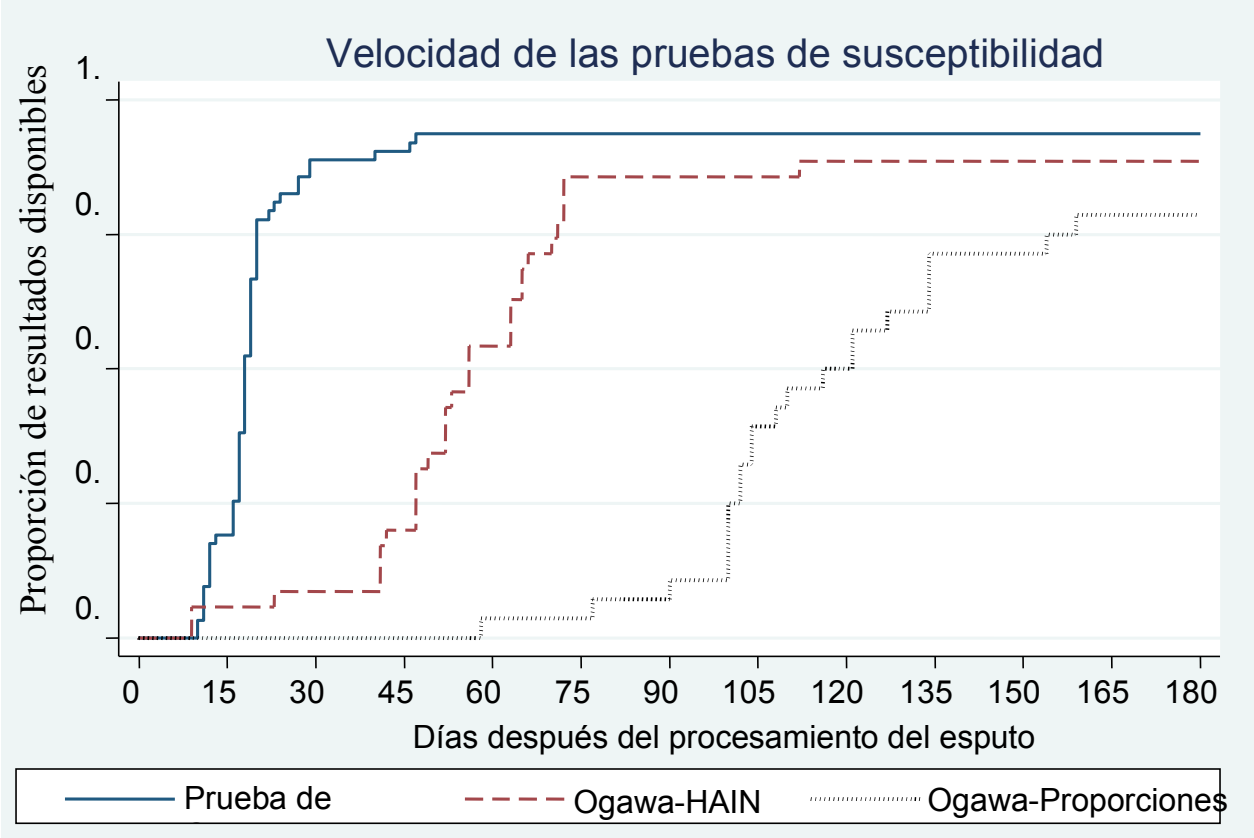
Antecedentes: El diagnóstico de TB-MDR en contextos de recursos limitados es un desafío ya que presentan limitada capacidad para realizar pruebas de sensibilidad a nivel local y a las demoras debido a envíos de las muestras a laboratorios referenciales distantes. Debido a ello, evaluamos la Prueba de Colores para TB MDR/XDR, una técnica en agar de capa delgada, en la cual se aplica el esputo contenido en el envase directamente a la placa de cultivo colorimétrica para luego ser sellada permanentemente, proporcionando asi, resultados de detección de TB y susceptibilidad a drogas al mismo tiempo .

Métodos: Se colectaron muestras de esputo (n=697) de pacientes con sospecha de TB pulmonar y que fueron enviadas al laboratorio de referencia local en la Amazonia Peruana. Este laboratorio estuvo equipado con una cabina de bioseguridad, una incubadora y un microscopio sin contar con otro equipo relacionado a diagnóstico de TB. El esputo fué procesado inmediatamente con el actual

algoritmo estandarizado usando la decontaminación modificada de Petroff y el cultivo de Ogawa (sin centrifugación). Los cultivos positivos de Ogawa posteriormente fueron enviados por aire al laboratorio referencial nacional para la realización de las pruebas de sensibilidad a drogas usando pruebas rápidas moleculares (1) con la prueba de HAIN y/o (2) de la prueba de cultivo tradicional con la técnica de proporciones. Adicionalmente para este proyecto de investigación, el esputo residual se añadió (3) a un desinfectante e inmediatamente se aplicó a la Prueba de Colores para TB MDR/XDR, se sello permanentemente y paso a la incubadora, todo el proceso se hizo localmente. Todos los cultivos fueron leídos semanalmente. El tiempo para los resultados de susceptibilidad a drogas fué comparado entre las tres aproximaciones diagnósticas.

Resultados: Los resultados de susceptibilidad fueron realizados en 63 muestras que fueron cultivos positivos para cualquiera de las pruebas. 87% (55/63) fueron cultivos positivos para Ogawa y todos tuvieron resultados de susceptibilidad a drogas disponibles por el Laboratorio Referencial Nacional. El tiempo desde el procesamiento del esputo hasta obtener los resultados tuvo una mediana de 54 días para el cultivo Ogawa seguido de la prueba HAIN y 112 días para la prueba de proporciones. La prueba de Colores para TB MDR/XDR para susceptibilidad realizada localmente, proporcionó resultados para el 94% (59/63) y el tiempo desde el procesamiento del esputo hasta los resultados de sensibilidad a TB, tuvo una mediana de 19 días, significativamente mas rápida que las otras pruebas (Figure).

Conclusiones: La prueba de susceptibilidad realizada localmente directamente del esputo con la prueba de Colores para Tb MDR/XDR fué exitosamente implementada in un laboratorio básico de la selva peruana y proporcionó resultados considerablemente más rápidos que el actual algoritmo diagnóstico que usa pruebas realizadas a distacia de cultivos positivos con técnicas moleculares rápidas.



Reference of your abstract and your poster board	PC-876-03
Title of your abstract	Comparison of time to MDR-TB diagnosis with the MDR/XDR-TB Colour Test & current algorithm in a resource-poor jungle setting in Peru
Type of session	Poster Discussion session
Title of session	53. MDR-TB diagnosis
Date, time and place	Sunday, 3 November 2013 from 12:45 to 13:45