Simplifying culture-based TB/MDRTB testing for peripheral labs

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.

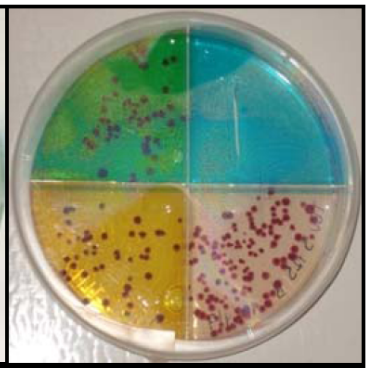
Novel Colour Test technique. On arrival in the laboratory, the contents of the sputum pot were applied directly without any processing to each quadrant of a culture plate. The culture plate contained a transparent thin-layer of 7H11 agar made selective with antimicrobials to prevent contamination and 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.

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 ($p=0.02$).

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.

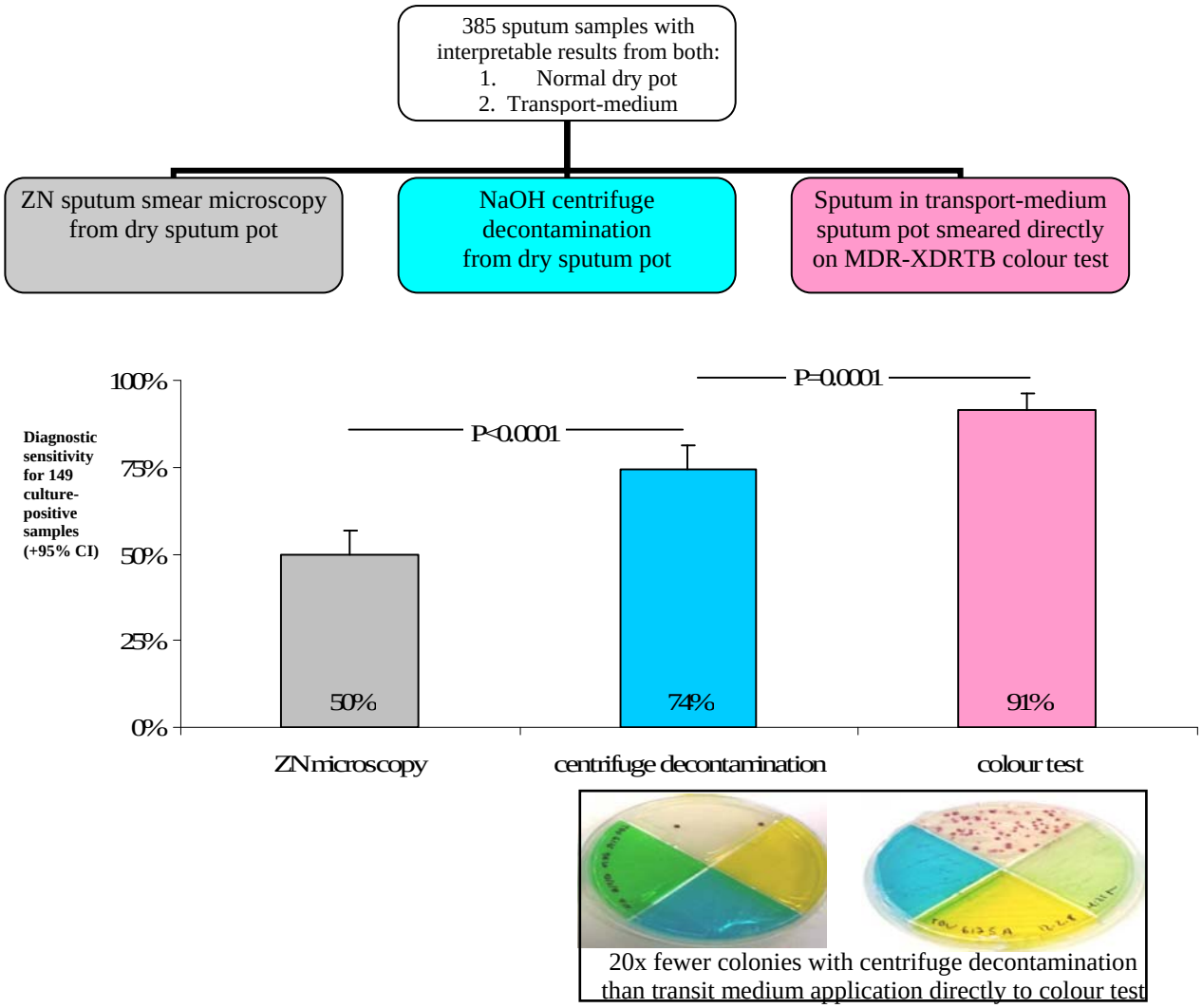
An MDR-XDRTB colour test for drug-susceptible TB that only grew in the clear detection quadrant (red dots), not the coloured antibiotic-containing quadrants.



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

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@lshtm.ac.uk

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]

[1]Universidad Peruana Cayetano Heredia, Lima, Perú; [2]Asociación Benefica Prisma, Lima, Perú; [3]Johns Hopkins Bloomberg School of Hygiene & Public Health, Baltimore, USA; [4]St. George's Hospital Medical School, London, UK; [5]London School of Hygiene & Tropical Medicine, UK.

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