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# Evaluation of the Kudoh swab method for the culturing of *Mycobacterium tuberculosis* in rural areas

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**Summary**

OBJECTIVE To compare the simple, swab 'Kudoh method' for culturing *Myobacterium tuberculosis* from sputum samples, to the standard Petroff digestion-decontamination procedure. The Kudoh method, which requires no centrifugation and takes only 4–5 min per sample, was also evaluated for its performance in a rural setting.

METHODS Two hospital laboratories in Caracas, Venezuela processed 314 sputum samples, in parallel, with both methods. Separately, sputum specimens were cultured with the Kudoh swab method in a field environment with minimal laboratory facilities.

RESULTS In the hospital laboratories, the sensitivity of the Kudoh swab method was comparable to that of the standard Petroff culture procedure. The swab method also performed satisfactorily in the field, improving the diagnostic sensitivity by 21% over microscopic examination alone.

CONCLUSION The Kudoh swab method is an acceptable alternative for culturing mycobacteria that is particularly suitable for rural laboratories lacking adequate infrastructure for the Petroff method.

**keywords** *Mycobacterium tuberculosis,* diagnosis, culture, Petroff, Kudoh swab method

## Introduction

Culturing of sputum specimens is now more important than in the past because of the increase in drug-resistant tuberculosis, the prevalence of atypical mycobacterial infections in HIV positive patients, and effective techniques for molecular epidemiology. In Venezuela, as in most countries, the sodium hydroxide method, also known as the method of Petroff (Kent *et al.* 1985), has been used as the standard digestion-decontamination procedure for sputum samples. This method is cumbersome though, as it requires at least 30 min for centrifugation and processing in a biological safety cabinet by technically skilled personnel. This is one of the important reasons that culturing of sputum samples, especially in rural areas with poorly equipped laboratories, is not common in Venezuela and most other developing countries.

Kudoh and Kudoh (1974) described a simplified swab method for culturing mycobacteria that takes only 3–4 min per sample, and needs neither technical skill, centrifugation nor a biosafety cabinet. In this method, sputum is gathered onto a cotton swab, the swab is decontaminated for 2 min in a solution of 4% sodium hydroxide, and then directly smeared onto a suitable culture medium. To our knowledge, there is only one study in the international literature that evaluated this method and compared it with the Petroff decontamination procedure (Kothadia & Sengupta 1982). The two methods gave similar results for both sensitivity and percentage of contaminated cultures but only smear positive sputum samples were processed with the two methods.

We evaluated the Kudoh swab culture method with smear positive and negative sputum samples in two hospital laboratories in Caracas, Venezuela and compared its performance with the traditional Petroff method. In addition, we also evaluated the swab method in a field setting without any laboratory infrastructure for culturing mycobacteria: the isolated hamlets of the Amerindian Warao people, who live in the delta of the Orinoco River.

## Material and methods

We collected 315 sputum samples from inpatients and outpatients at two Caracas hospitals, José Gregorio Hernández and José Maria Vargas. Only sputum samples with a volume of 2 ml or more were included in the study. Samples were labelled as saliva, mucoid, mucopurulent or purulent according to their physical appearance, and processed in a biosafety cabinet for acid-fast staining and both Kudoh and Petroff methods.

During visits in the Orinoco River Delta, 651 sputum samples were collected from Warao Amerindians living in

widely dispersed communities throughout the Orinoco River Delta. These samples were processed both for acid-fast staining and for culture with the Kudoh swab method in the communities where they were obtained, without any laboratory facilities. The inoculated culture tubes were transported to the laboratory for growth.

For the acid-fast smears, sputum specimens were smeared onto glass slides for microscopic examination prior to processing the sample for culture. The slides were then taken to a rudimentary laboratory in one of the largest Warao communities, where they were heat fixed, stained with the Ziehl–Neelsen technique and then examined under oil immersion (x 1000). Smears were classified as negative only when at least 100 fields were examined without finding acid fast bacilli (Kent *et al.* 1985).

For culture, the recommended standard Petroff procedure was used (Kent *et al.* 1985). Briefly, 2 ml of sputum was mixed in a test tube with 2 ml of a 4% sodium hydroxide solution with 0.04 g/l phenol red and incubated for 15 min at room temperature. The test tube was centrifuged for 20 min at 2500 *g* and the sediment was neutralized with 1 N HCl. The neutralized sediment was inoculated into two tubes containing Lowenstein–Jensen medium.

The Kudoh swab method was performed as reported by Kudoh and Kudoh (1974). Sputum was picked up onto a sterile cotton swab, and the swab was immersed in a sterile 4% sodium hydroxide solution in a test tube. After 2 min the swab was removed from the test tube and the specimen was directly inoculated into two tubes containing modified Ogawa culture medium (pH 6.4) by smearing and squeezing the swab over the surface of the media (Figure 1). Cultures were kept for 6 weeks at 37 °C and were reported positive if five or more colonies grew on one or more tubes. Slides for microscopy were prepared from colonies growing on the culture media, and if AFB were found the colony was subcultured onto Lowenstein-Jensen medium for species identification using biochemical methods [Niacin production and nitrate reductase activity according to Kent *et al.* (1985)].

Fischer's exact test was used to evaluate the differences between the results of cultures processed with the Kudoh swab and Petroff methods.

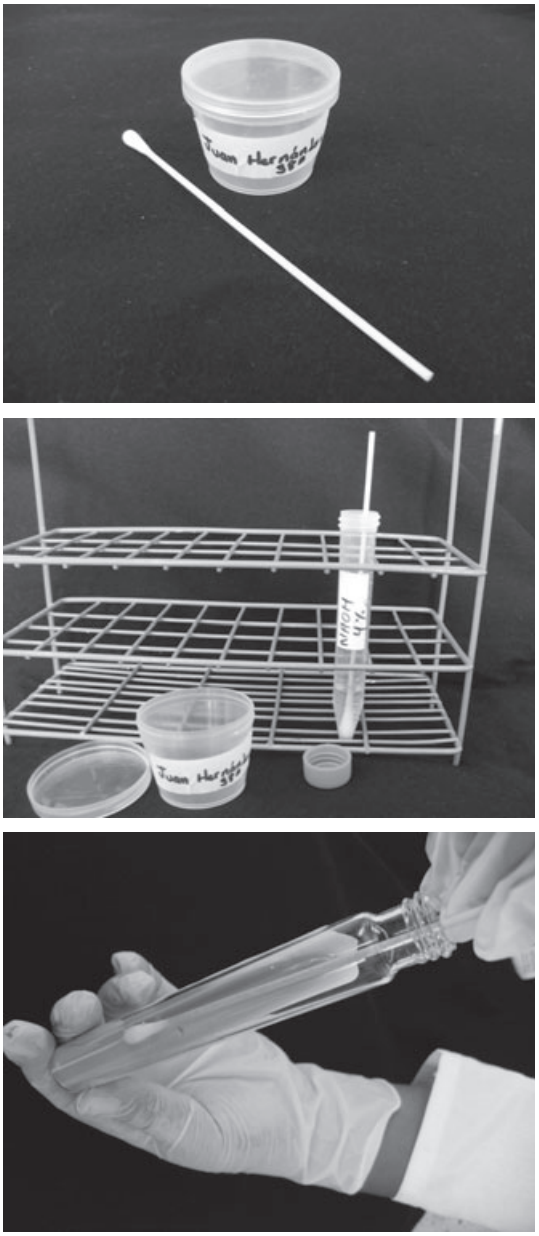

**Figure 1** Visualization of the Kudoh swab method for the culturing of *Myobacterium tuberculosis* from sputum samples. Step 1. Capture a purulent part of the sputum specimen onto a sterile cotton swab. Step 2. Place the swab in a tube containing approximately 2 ml of a 4% solution of sodium hydroxide, and leave immersed for 2 min. It is critical that the cotton part of the swab be completely submerged in the sodium hydroxide solution. When withdrawing the swab, take care that it does not touch the walls of the tube, which are a source of contamination. Step 3. Inoculate the sample directly by smearing and squeezing the swab onto a slant containing Lowenstein-Jensen or modified Ogawa media. Incubate the tubes at 37 °C.

## Results

In two hospital laboratories, 315 sputum samples from symptomatic patients were processed in a safety cabinet for acid-fast staining and for culture using both the Petroff and Kudoh methods. Of these 315 samples, 77 were positive for TB: 56 were positive by both acid-fast staining and culture, 21 were positive only with one or both culture

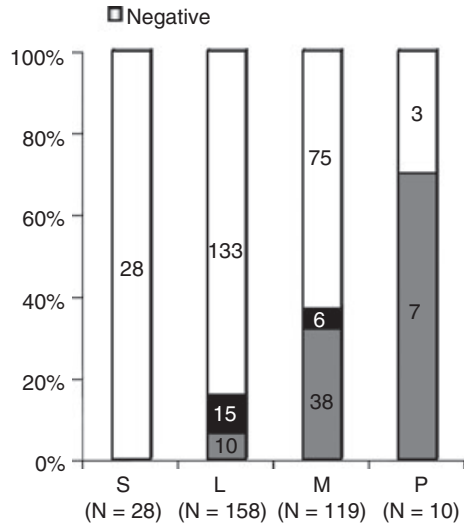


**Figure 2** Relation between quality of the samples and a positive diagnosis for tuberculosis. Sputum samples received in both hospitals were classified according to their quality as saliva (S), mucoid (L), mucopurulent (M) and purulent (P) positivity of these samples for both smear staining and culture (grey) and for culture only (black) are indicated in the respective bars.

methods. All isolates were identified as *Mycobacterium tuberculosis* with molecular techniques (spoligotyping). Thus, sputum cultures improved the sensitivity of the diagnosis by 37.5%. Although 186 of the 315 samples (59%) were considered of poor quality, being either saliva or mucoid, culturing improved the diagnosis in mucoid specimens by 150% (10 positive by smear and culture, 15 positive by culture alone (Figure 2).

The sensitivity of both culture methods was not significantly different (Fisher, $P = 0.7473$). Of the 77 positive samples, 68 (88.3%) were culture positive with the Petroff method and 71 (92.2%) with the Kudoh method. The two culture methods showed no difference in sensitivity with smear negative samples or in the number of colonies recovered. Smear positive sputum samples always resulted in more than 100 colonies per culture tube, while smear negative samples yielded between 10 and 100 colonies per tube. In our setting, samples processed with the Kudoh method where significantly less contaminated (3.7%) than samples processed with the Petroff method (7.3%) (Fisher, $P = 0.0072$). There was no difference between the two methods in the time required for the colonies to become visible (results not shown).

We used the Kudoh method to diagnose TB in the Warao Amerindian population from the Orinoco River Delta. The inoculation of cultures employing traditional methods is virtually impossible in this setting, as the communities are far from even the inadequate laboratories present in the region. During five expeditions, 651 sputum samples were processed, of which 38 were smear positive/culture positive and eight were smear negative/culture positive. Thus cultures with the Kudoh method improved the sensitivity of tuberculosis diagnosis by 21%. Cultures from 51 sputum samples were contaminated (7.8%), which, given the rate of smear negative, culture positives in the non-contaminated 600 specimens, translates into less than one tuberculosis patient not diagnosed. This should be considered acceptable given the conditions in which the cultures were inoculated.

## Discussion

We confirmed that sputum sample culture is significantly more sensitive than the direct smear for the diagnosis of pulmonary tuberculosis, especially with mucoid and mucopurulent specimens. About 60% of the sputum samples received in both hospital laboratories were saliva or mucoid specimens (Table 1) showing the importance of instructing the patient on how to produce adequate sputum specimens as distinct from purely saliva.

The Kudoh swab method was as sensitive as the Petroff decontamination procedure in the diagnosis of pulmonary TB, and thus represents a valuable alternative method for culturing mycobacteria that is especially appropriate for rural laboratories without the proper infrastructure for traditional culturing. The method needs no laboratory equipment and, because of the minimal manipulation of the sample, has a low biosecurity risk. Of course transport, manipulation and subsequent disposal of the cultures must be carried out with appropriate precautions in a suitably equipped laboratory. The simplicity and safety of the Kudoh method allows for rapid and accurate culturing of sputa at field sites, but in traditional laboratories with a high sample volume it could alleviate the work load by eliminating the time-consuming centrifuge step. Most importantly, we have demonstrated that the method permits the recovery of clinical isolates in areas where traditional culturing is impossible. In the Orinoco Delta, without any laboratory facility, the sputum samples were frequently processed for culture in the open air. In our experience the Kudoh swab method is easy to master, even by personnel without any experience in the microbiology of tuberculosis. We believe that the Kudoh method could be an alternative method for low-resource countries with limited technical resources to provide culture methods for priority needs such as drug resistance surveillance, extrapulmonary and childhood and MDR-TB.

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