EVALUATION OF AN OGAWA *MYCOBACTERIUM* CULTURE METHOD MODIFIED FOR HIGHER SENSITIVITY EMPLOYING CONCENTRATED SAMPLES

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Abstract: Two egg-based culture media were evaluated for detection of mycobacteria with Lowenstein-Jensen (L-J) as a gold standard. The conventional culture method was modified to improve laboratory diagnosis of tuberculosis in resource scarce countries by employing an inexpensive but sensitive and specific culture method. Sputum samples were collected from pulmonary tuberculosis suspects who visited the chest clinic at the University Teaching Hospital in Zambia. These samples were processed using three different sample treating procedures (with or without sample concentration) and cultured on L-J and Ogawa media for mycobacteria isolation. A total of 276 sputum samples were collected from 138 pulmonary tuberculosis suspects. When the L-J result was used as a standard, the sensitivity of Ogawa and modified Ogawa was 81.7% and 90.3% respectively. Similarly, the specificities of those methods were 96.7% and 92.3% respectively. In total, 90 samples (32.6%) were smear positive and 108 (39.1%) were culture positive. The positivity of each culture method was as follows: 93 (33.7%) in L-J, 98 (35.5%) in modified Ogawa, and 82 (29.7%) in original Ogawa. The contamination rate was 1.1%, 5.1%, and 9.8% for L-J, Ogawa and modified Ogawa respectively. The Ogawa culturing method is economical, simple and quick. Its low sensitivity was overcome by employing the concentration method, the sensitivity significantly improving from 81.7% to 90.3%. Ogawa techniques are ideal in overburdened TB laboratories with poor resources in developing countries.

Key words: Ogawa, culture, tuberculosis, and developing country

INTRODUCTION

Tuberculosis is still one of the main public health problems in most developing countries. Global estimations indicate that approximately 8 million new cases of active tuberculosis are notified every year and that 2 million people die of tuberculosis every year (Davies et al., 1996). Incidences are rising in most parts of the world, especially in developing countries where the epidemic of human immunodeficiency virus (HIV) has had the effect of increasing the number of tuberculosis cases. These countries, apart from facing an increased incidence of tuberculosis, are also overburdened with other HIV and non-HIV related problems including depressed economies from drought, famine and the effects of a worldwide recession (Mwinga, 1994). In Zambia, tuberculosis (TB) is one of the major causes of mortality among adults, accounting for 13% of all adult deaths (ZAMBART, 1999). Incidence of TB countrywide has risen five fold from 100 per 100,000 population in 1980 to approximately 500 per 100,000 population in 1996, making Zambia a country with one of the highest incidences of TB in the world (NASTL, 1996; NTP, 1996; WHO, 2002).

The conventional microscopy for acid-fast bacilli (AFB), which 99% of the country's health services provide, is rapid in obtaining results but it has a relatively low sensitivity. The culture method with egg-based media is a conventional way of isolating mycobacteria, but it is time consuming. Recently, the results of radiometric and non-radiometric BACTEC/MGIT systems demonstrated that they are more efficient in the recovery of mycobacteria (Zanetti et al., 1997). However, they are far too expensive for most developing countries. L-J is the standard method for the culture of mycobacteria in Western countries. Ogawa was developed as an inexpensive alternative but proved to have a relatively low sensitivity as compared to L-J (Ogawa et al., 1960). Thus, the Ogawa method employing concentrated samples (modified Ogawa) may be a better alternative even though it may produce a higher contamination rate. In this study we compared the sensitivity of Ogawa and modified Ogawa cultures with that of L-J to evaluate the usefulness of the modified Ogawa method.

MATERIALS AND METHODS

Clinical samples

One hundred and thirty eight patients with suspected pulmonary tuberculosis visiting the chest clinic at the University Teaching Hospital in Lusaka, Zambia submitted 2 early morning sputum samples consecutively. Smears were prepared by taking a purulent portion of the sputum and smearing it on labelled slides. The slides were air-dried, heat fixed and stained by the standard auramine-phenol method. These smears were examined by fluorescent microscopy (x200). All procedures were performed by one designated technologist.

Culture methods

Each medium was prepared to standard protocol. (Table 1) For the L-J method, the sputum samples were decontaminated by adding 3 volumes of 4% sodium hydroxide (NaOH) to one volume of sputum and incubating at 37 °C for 15 minutes. They were then centrifuged at 3,000 g for 15 minutes. After disposing the supernatant by decantation,

approximately 10 ml of distilled water was added to neutralise the sodium hydroxide. The samples were centrifuged again under the same conditions and the supernatant was decanted. Exactly 0.2 ml of deposit was inoculated onto L-J slope. The inoculated slopes were incubated at 37 °C until growth was observed or for up to 8 weeks.

For the Ogawa method, the samples were decontaminated and homogenised by mixing one volume of sputum with 3 volumes of 4% sodium hydroxide and incubated for 15 minutes. Then, 0.2 ml of mixture was inoculated onto 3% Ogawa slopes. The inoculated slopes were incubated at 37 °C until growth was observed or for up to 8 weeks.

Modified Ogawa method

The clinical samples were decontaminated and homogenised in the same way as mentioned above. They were centrifuged at 3,000 g for 15 minutes and the supernatant was disposed by decantation. Then 0.2 ml of deposit was inoculated onto 3% Ogawa slopes without neutralisation. The inoculated slopes were incubated at 37 °C until growth was observed or for up to 8 weeks.

Reading of cultures

Cultures were read once a week for any growth. The culture tubes that showed positive growth were removed for identification and report. The contaminated cultures were removed and recorded.

Statistical analysis

The sensitivity, specificity, and positive and negative predictive values were compared among original 3% Ogawa, modified Ogawa and L-J cultures. The values for each method with their 95% confidence intervals (CI) were calculated using EpiInfo version 6.03.

RESULTS

A total of 276 samples from 138 pulmonary tuberculosis suspects were examined. With regard to smear results, 186 (67.4%) were negative but 90 (32.6%) were positive. The breakdown of positives was 14 (5.1%), 12 (4.3%), 3 (1.1%) and 61 (22.1%) for scant, 1 +, 2 + and 3 + (WHO standard) respectively. As for smear negative samples, eight patients showed discrepant culture results between the two consecutive samples. Six of the eight patients had a few colonies of positive cultures on each tube while the other two showed higher positives. No patient showed discrepant culture results in a pair of smear positive samples. Among the 90 smear positives, 80 (88.9%) samples were culture positive, while 28 (15.1%) of 186 smear negative samples were culture positive.

A total of 108 (39.1%) samples were found culture positive overall. In the culture positives, 93 (33.7%), 82 (29.7%) and 98 (35.5%) were detected by the L-J, original

Figure 1. The interrelation of culture results among the three methods

Table 1. Composition of L-J and Ogawa medium

Component	L-J	Ogawa
Potassium dihydrogen phosphate	1.2 g	9.0 g
Magnesium sulphate	0.12 g	-
Magnesium citrate	0.3 g	-
Sodium glutamate	-	3.0 g
L-asparagine	1.8 g	-
Glycerol/Sodium pyruvate	6.0 ml/3.6 g	18.0 ml/ -
Distilled water	300 ml	300 ml
Malachite green (2%)	16 ml	18 ml
Egg homogenate	500 ml	600 ml
Benzyl penicillin (1,000,000 IU/ml)	1 ml	-

The above measurements were conducted on 100 tubes of each type of media, each 12 ml capacity tube containing 7 ml of media. The mineral salts were autoclaved at 115 °C for 15 minutes. The egg homogenate and benzyl penicillin (as for L-J) were added aseptically after cooling to about 40 °C. The media was then coagulated at 90 °C for 60 minutes after dispensing.

Table 2. Comparison of sensitivity of Ogawa with the modified Ogawa and Lowenstein-Jensen method on the basis of sample number

		L-J method		
		Positive	Negative	Total
Ogawa	Positive	76	6	82
	Negative or contamination	17	177	194
	Total	93	183	276
Modified Ogawa	Positive	84	14	98
	Negative or contamination	9	169	178
	Total	93	183	276

3% Ogawa and modified Ogawa method respectively. (Table 2) The isolation sensitivity was not significantly different among these three methods. Seventy-two samples (26.1%) were positive in all three methods. Five samples were culture positive by the L-J method only with contaminations of other methods. One and 9 samples were positive only by 3% Ogawa and modified Ogawa respectively. Four samples were positive by both the L-J and 3% Ogawa method. Twelve samples were positive by the L-J and modified Ogawa method, and another 5 were positive by 3% and modified Ogawa method. The interrelation is shown in Figure 1. In total, the discrepant culture results were obtained in 54 of 276 (19.6%) samples tested using these three methods. If the contamination is included into negative culture results, the discrepancy was 8.0% (22/276).

The contamination rates were 1.1% (3 of 276), 5.1% (14 of 276) and 9.8% (27 of 276) in the L-J, 3% Ogawa and modified Ogawa method respectively. There were statistically significant differences between the L-J, and the 3% and modified Ogawa methods ($p = 0.0114$ and $p < 0.0001$ respectively). There was no difference between 3% Ogawa and modified Ogawa ($p = 0.0514$). The major contaminant was fungus. There was no tendency to find contaminated results in the samples treated on a specific day.

DISCUSSION

In this study, we evaluated three culturing methods with two egg-based solid media for the detection of *M. tuberculosis*. Despite the high incidence of tuberculosis (WHO, 2002), the developing countries in Sub-Saharan African region have few diagnostic centres that employ culture techniques in the detection and recovery of mycobacteria from clinical samples. In Zambia, for example, there are only two centres that employ culture techniques in the diagnosis of tuberculosis. In these countries, the lack of culture services in the diagnosis of tuberculosis can be attributed to the scarcity of medical resources.

The L-J method has been the standard technique of re-

covering mycobacteria from clinical samples in African countries. The L-J method requires three steps for sample preparation, i.e. decontamination, concentration, and neutralisation. It takes approximately 30 minutes for completion. On the other hand, the Ogawa method takes approximately half the time of L-J for sample preparation with decontamination only. This means that Ogawa is simple and quick. Additionally, from the aspect of economy, Ogawa is less expensive than L-J because of it requires few ingredients. However, the major disadvantage of Ogawa is its relatively low sensitivity due to the non-concentration of samples. The sensitivity of smear and culture for the detection of mycobacteria is improved by sample concentration (Apers et al., 2003). But, there has been no attempt to introduce the sample concentration step to the Ogawa method. In the present study, the sensitivity of Ogawa was improved by adopting the concentration step in the procedure of sample preparation. The improved sensitivity of Ogawa method was equal to or higher than that of the L-J method. It proved effective to employ the sample concentration procedure as a way of increasing the sensitivity of the Ogawa method, and this modification should be adaptable to the conventional Ogawa method without any complication.

The disadvantage of sample concentration in the Ogawa method as compared to the conventional method is the increased possibility of contamination. The contamination rate was not significantly different between the conventional and modified Ogawa method, although it was higher in the 3% Ogawa and modified Ogawa methods as compared to the L-J method. The higher rate of contamination may be attributable to the shorter exposure time to sodium hydroxide for decontamination because no neutralisation of sodium hydroxide is required in the Ogawa procedure. The non-use of penicillin will also contribute to the higher rate of contamination in the 3% Ogawa and modified Ogawa method as compared to the L-J. Additionally, the contaminated samples were mainly seen in one batch of samples. Some reagents might be contaminated by fungi, although no contamination was seen in the L-J at the same period. Another possible, probably major, reason is that the technologist was not practiced in the Ogawa method because the Ogawa method is not implemented widely in African countries. However, the technologists are familiar with the L-J method, which requires the concentration step for sample preparation. The contamination rate will undoubtedly be reduced by further practice. Therefore, when a laboratory attempts to introduce the modified method, avoiding additional workload and cost, it is advisable to employ the method as indicated in this study. It is also advisable to introduce this method to a new laboratory because of its simplicity.

Several recent reports have highlighted the low positivity of sputum examination in HIV positive patients. Zambian tuberculosis patients also showed low sputum smear positivity in HIV positive patients in past studies (Elliott et al., 1993 and Johnson et al., 1997). The implementation of the culture method will increase the isolation of mycobacteria, without any advanced technologies even in HIV epidemic areas. Then, it is important to choose an economical and sensitive culturing method.

A definitive diagnosis of tuberculosis is still dependent on the isolation of *Mycobacterium tuberculosis* by culturing. Although the culture on solid media is inexpensive, it is time consuming and low sensitive (Somoskovi and Magyar, 1999). Although the liquid medium-based radiometric and non-radiometric systems have reduced the recovery time of mycobacteria and have improved the sensitivity of recovery, the cost of these systems is beyond the reach of most developing countries (Pfyffer et al., 1997 and Pfyffer et al., 1997). As a compromise, a slightly cheaper egg-based culture technique, the modified Ogawa, could be employed in the routine diagnosis of tuberculosis in developing countries where resources are scarce.

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