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NIACIN PRODUCTION TEST IN MYCOBACTERIA: REPLACEMENT OF BENZIDINE-CYANOGEN BROMIDE REAGENT BY o-TOLIDINE-CYANOGEN BROMIDE

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Introduction

The identification of *M. tuberculosis* depends primarily on the niacin production test. Runyon and others (1959) described a method based on the observations of Konno (1956) using aniline as the reagent. However, the aniline reagent gives a yellow colour which can cause difficulty in the interpretation of the results, particularly in the case of the chromogenic mycobacteria. Hence several workers prefer the test employing benzidine (Medveczky, 1960) or o-tolidine (Guttierrez-Vazquez, 1960), since the pink colour produced in these tests is easier to read.

The standard method for niacin production test at this Centre has been the one using benzidine. However, satisfactory supplies of benzidine are no longer available, as the manufacture of this compound' has recently been stopped. Hence it was decided to investigate the test using o-tolidine. Though other workers (Tarshis, 1960, 1961; Gangadharam and Droubi, 1971) have compared the benzidine and o-tolidine methods on small numbers of cultures, no large scale investigation of these two methods has been reported. Therefore a direct controlled comparison of these two methods was undertaken, the results of which are reported here.

Material and Methods

A total of 560 cultures of mycobacteria was used for this comparison. These cultures formed part of a survey.

A standard suspension was prepared from each culture by shaking it with sterile distilled water and glass beads. One loopful of this suspension was inoculated on to a pair of Lowenstein-Jensen slopes and incubated at 37°C. At the end of four weeks, the two sets were given code numbers and processed. The investigation was carried out in three batches, using 150-200 cultures per batch. Both the tests were performed and read by the same person.

Benzidine test –To approximately 0.25 ml of the autoclaved culture extract was added 0.25 ml of a freshly prepared 3% w/v solution of benzidine (E. Merck, GR) in ethanol followed by an equal volume of approximately 10 % cyanogen bromide (saturated aqueous solution).

o-tolidine-test – The procedure was essentially similar to that of the benzidine test except that the benzidine was replaced by a freshly prepared 15 % w/v solution of o-tolidine (BDH Analar) in ethanol.

With both the tests, the formation of a pink or red precipitate was considered to be a positive reaction for niacin while a white or dirty-white precipitate was taken as a negative reaction. The positive results were graded as 1 + (faint perceptible pink precipitate) or 2+ (pink or red precipitate).

Results and Conclusions

Of the 560 cultures tested (Table 1) 174 were negative and 380 were positive by both tests, that is. an agreement of 99 %. Of the remaining six specimens, 4 yielded a positive reaction only by the o-tolidine method (1 was 1 + and 3 were 2+), and 2 by the benzidine method only (both 1+). It may be concluded that the efficiency of the o-tolidine method is very similar to that of the benzidine method in detecting niacin production.

Table 1

Comparison of the benzidine and o-tolidine methods for the detection in niacin production in mycobacteria

Benzidine method	o-tolidine method			Total
	Neg.	1 +	2 +	
Neg.	174	1	3	178
1 +	2	1	0	3
2 +	0	6	373	379
Total	176	8	376	560

Summary

The benzidine and o-tolidine methods for niacin production were compared on 560 cultures. There was an excellent agreement (99%) between the two methods.

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