

DIRECT SENSITIVITY TEST FOR ISONIAZID

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INTRODUCTION.

It is well known that the results of isoniazid sensitivity tests by the indirect method have prognostic significance in the treatment of tuberculosis with regimens containing isoniazid (Tuberculosis Chemotherapy Centre, Madras, 1960 ; Devadatta *et al.*, 1961) ; the indirect method is, however, complex and time-consuming, and therefore not feasible in institutions with limited facilities.

Direct sensitivity tests are attractive because they involve only one stage of handling (i.e. cultures need not be set up), are simpler and less expensive, and consume less time. Although the direct sensitivity test introduced by Middlebrook and co-workers (Middlebrook and Cohn, 1958 ; Russell and Middlebrook, 1961) using 7H10 agar medium yielded satisfactory results, it has certain disadvantages. Thus, the medium employed is expensive, the ingredients are not readily available in this country and, in our experience at Madras, losses due to contamination can be considerable.

This paper describes a direct sensitivity test for isoniazid using Lowenstein-Jensen medium, and compares the findings obtained by this method with those obtained by the indirect method.

MATERIALS AND METHODS.

Sputum specimens.— In all, 486 sputum specimens were collected from the same number of tuberculous patients, all of whom were in an ICMR co-operative investigation (Indian Council of Medical Research Second Drug Resistance Investigation 1969). The specimens were obtained over a period of 6 months at 9 Centres in India and transported by air to central laboratory at Madras.

Smear and culture examination.— Direct smears were examined by fluorescence microscopy (Holst, Mitchison and Radhakrishna, 1959). For culture, the sputum specimen was treated with 4 per cent sodium hydroxide for 20 minutes and then centrifuged for 15 minutes, after addition of distilled water to the deposit, the specimen was centrifuged again, and the deposit inoculated with a 5-mm. loop on to 2 slopes of Lowenstein-Jensen medium without potato starch (Jensen, 1955), and incubated at 37°C. The slopes were examined weekly, and reported as negative if no growth was present by 8-9 weeks.

Indirect sensitivity tests.—As soon as the culture became positive, an indirect sensitivity test was set up. Using a 22 SWG nichrome loop, approximately 2 mg.

(moist weight) of bacilli; as judged by eye, were taken from the positive culture and added to a 4 oz. screw-capped bottle containing 5-mm. beads and 0.5 ml. sterile distilled water. A suspension was prepared by shaking the bottle for 1 minute in a mechanical shaker. Using a 3-mm. external diameter loop (27 SWG), a loopful of the suspension was spread on the surface of each of 5 slopes of Lowenstein-Jensen medium, namely, those containing pre-inspissation concentrations of 0, 0.2, 1, 5 and 50 $\mu\text{g./ml.}$ of isoniazid. The standard sensitive strain, H37Rv, was set up with each batch of tests. The slopes were read at the end of 4 weeks of incubation at 37°C.

Direct sensitivity test.— The deposit left over in the bottle after setting up the culture was inoculated on to 4 slopes of Lowenstein-Jensen medium with a 5-mm. loop. Two of these contained a pm-inspissation concentration of 0.2 $\mu\text{g./ml.}$ of isoniazid and the remaining 2 slopes, which were drug-free, served as controls. All the 4 slopes were incubated at 37°C. and were read at 1, 2, 3, 4, 6 and 8 weeks. Growth typical of *Mycobacterium tuberculosis* was recorded as 3-plus if it was confluent, Z-plus if there were more than 100 colonies, and 1-plus if there were 100-20 colonies; the number of colonies was recorded if it was less than 20. After each reading, the racks were replaced in the incubator.

DEFINITION OF RESISTANCE.

Indirect sensitivity test.—(a) Growth of 20 or more colonies, on the 1 $\mu\text{g./ml.}$ slope, or (b) growth of 20 or more colonies on the 0.2 $\mu\text{g./ml.}$ slope, but not on the 1 $\mu\text{g./ml.}$ slope, followed by growth of 20 or more colonies on the 0.2 $\mu\text{g./ml.}$ slope in a repeat test.

If the control (drug-free) slope yielded less than 100 colonies, the result was ignored and the test repeated.

Direct sensitivity test.— Two definitions were used, the first based on the findings of the two 0.2 $\mu\text{g./ml.}$ slopes and the two controls, and the other based on the first 0.2 $\mu\text{g./ml.}$ slope and the two controls.

Two-slope definition.—(a) Growth of 20 or more colonies on one (or both) of the slopes containing 0.2 $\mu\text{g./ml.}$ of isoniazid, or (b) growth of 1 to 19 colonies on *each* of the two slopes containing 0.2 $\mu\text{g./ml.}$ of isoniazid, provided that the growth was 100 colonies or less on at least one of the drug-free slopes.

One-slope definition.—(a) Growth of 20 or more colonies on the first slope containing 0.2 $\mu\text{g./ml.}$ isoniazid, or (b) growth of 1 to 19 colonies on the first slope containing 0.2 $\mu\text{g./ml.}$ of isoniazid, provided the growth was 100 colonies or less on at least one of the drug-free dopes.

RESULTS.

Losses due to culture negativity or contamination.— Loss due to culture negativity (in the case of the direct test, absence of growth on the drug-free and the drug-containing slopes) or contamination occurred in similar proportions with the indirect method **and** the direct method employing 2 slopes, and were of the order of 25 per cent.

However, the proportion increased to about 30 per cent when the first slope only was considered in the direct test.

Comparison of the results of direct and indirect tests.- In the direct test, the percentage of specimens with growth on the drug-free slopes was not high in the first 3 weeks ; consequently the comparisons are presented only from the fourth week onwards.

Two-slope definition.- Table I presents the comparisons (at 4, 6 and 8 weeks) between the direct sensitivity test method using the two-slope definition and the indirect sensitivity test method.

TABLE 1.
Classification of cultures as isoniazid-sensitive or isoniazid-resistant by the indirect and Direct sensitivity test methods (two-slope definition).

ISONIAZID SENSITIVITY:		DIRECT TEST RESULT READ AT :					
Indirect test.	Direct test.	4 weeks.		6 weeks.		8 weeks.	
		Number of cultures.	Per cent.	Number of cultures.	Per cent.	Number of cultures.	Per cent.
Sensitive	Sensitive	247	} 94	252	} 94	249	} 93
Resistant	Resistant	70		74		75	
Resistant	Sensitive	13	} 4	10	} 3	9	} 3
Sensitive	Resistant	9		11		14	
Total		339	101	347	100	347	100

The classification (as isoniazid-sensitive or isoniazid-resistant) based on the direct test result at 4 weeks was identical with that based on the indirect test result for 94 per cent of 339 specimens. Of the remaining 22, 13 were classified as resistant by the indirect test but sensitive by the direct test, and conversely, 9 were classified as sensitive by the indirect but resistant by the direct test ; the difference was non-significant ($P > 0.2$). At 6 and 8 weeks, the findings with the indirect test were very similar, there being no further increase in the percentage of agreement between, the two tests.

Analyses (not tabulated here) were carried out separately for the smear-negative and the smear-positive specimens. In the smear-negative specimens, the agreement between the methods was 89, 91 and 91 per cent of 45 specimens at 4, 6 and 8 weeks, respectively. In the smear-positive specimens, it was 94 per cent of 294 at 4 weeks, and 94 percent of 302, both at 6 and at 8 weeks.

One-slope definition.- Table II presents the comparisons between the direct test using the one-slope definition and the indirect test. At 4 weeks, the classification was the same by both methods for 94 per cent of 323 specimens. Considering the **disagreements**, 15 cultures were as resistant by the indirect test but sensitive by the direct test, while only 4 cultures were **classified as sensitive by the indirect test,**

but resistant by the direct test—a significant difference ($P=0.02$). ~~Thus~~ there was some evidence at 4 weeks of the direct test under-estimating the ~~proportion~~ with isoniazid resistance. However, this feature was less clear at 6 weeks ($P > 0.2$) and had totally disappeared by 8 weeks. Even so, there was no appreciable increase in the extent of agreement between the two methods, the proportion with agreement being 95 per cent at both 6 and 8 weeks.

TABLE II.

Classification of cultures as isoniazid-sensitive or isoniazid-resistant by the Indirect and Direct sensitivity test methods (one-slope definition).

ISONIAZID-SENSITIVITY :		DIRECT TEST RESULT READ AT :					
Indirect test.	Direct test.	4 weeks.		6 weeks.		8 weeks.	
		Number of cultures.	Per cent.	Number of cultures.	Per cent.	Number of cultures.	Per cent.
Sensitive	Sensitive	239	94	241	95	239	95
Resistant	Resistant	65		70		73	
Resistant	Sensitive	15	5	11	3	8	2
Sensitive	Resistant	4	1	6	2	8	2
Total		323	100	328	100	328	99

Analyses (not tabulated here) of the smear-negative specimens showed that the proportions with agreement were 86, 89 and 91 per cent of 44 specimens at 4, 6 and 8 weeks, respectively. For the smear-positive specimens, the corresponding proportions were 95 per cent of 279 at 4 weeks, and 96 per cent of 284, both at 6 and at 8 weeks.

DISCUSSION.

The findings with the direct isoniazid sensitivity test described in this paper are very encouraging. Thus, at 4 weeks, the extent of agreement (in classifying strains as isoniazid-sensitive or isoniazid-resistant) between the direct test and the standard indirect sensitivity test was 94 per cent. This order of agreement is only slightly less than that obtained (97 per cent) when the indirect sensitivity test is repeated on the same. culture (Gangadharam, 1965).

The extent of agreement between the two methods was slightly less in smear-negative specimens, but still of a high order, namely, about 90 per cent. Finally, there was little evidence of decrease in the extent of agreement if only one slope of 0.2 $\mu\text{g./ml.}$ of isoniazid was considered, instead of two ; however, there was tendency for the test to under-estimate resistance ; furthermore, losses due to contamination or failure of growth increased by about 5 per cent.

Comparisons of the direct and indirect isoniazid sensitivity. tests in Lowenstein-Jensen medium have been reported by Mackey (1964) and, more recently; by Stewart,

and Burnet (1968). In Mackey's investigation, which was based on 783 specimens the direct test yielded 'indeterminate' results for 33 per cent of the specimens, mainly due to sparse growth on drug-free medium or to contamination. In the remaining specimens, however, the agreement between the two methods was highly satisfactory, namely 98 per cent. In the investigation reported by Stewart and Burnet, only 31 specimens were studied and the agreement found to be 90 per cent.

SUMMARY.

A direct isoniazid sensitivity test, employing Lowenstein-Jensen medium, is described. A controlled comparison, based on 486 sputum specimens, with the indirect isoniazid sensitivity test showed that the extent of agreement between the two methods was 94 per cent.

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