Correspondence

A SIMPLE AND RAPID COLD-STAINING METHOD FOR ACID-FAST BACTERIA

To the Editor of the American Review of Respiratory Diseases:

During the course of our experiments with acid-fast microorganisms, a new staining method for acid-fast bacteria was developed based on a combination of the Kinyoun and Gabbett staining techniques.

The acid-fast bacteria stood out clearly in infected specimens and were in no way inferior to the well-known staining methods of Ziehl-Neelsen, Kinyoun, or Gabbett.

The staining solutions are very easy to prepare and it has been found that the solutions revealed after three months at 27°–30°C. no deterioration or variation in staining ability.

This new cold-staining method equals other staining methods of Ziehl-Neelsen, Kinyoun, or Gabbett in rapidity and ease, since the new technique requires only four steps in just 4½ minutes instead of eight steps to be carried out in 8 minutes by the Ziehl-Neelsen technique.

Details on the new staining procedure are as follows:

The smear is covered with Kinyoun's carbol-fuchsin (basic fuchsin, 4 gm.; phenol, 8 ml.; 95 per cent alcohol, 20 ml.; distilled water, 100 ml.) and allowed to stand for three minutes. It is then washed with tap water (one half minute), following which the slide is then flooded with Gabbett's solution (methylene blue, 1 gm.; 96 per cent sulfuric acid, 20 ml.; absolute alcohol, 30 ml.; distilled water, 50 ml.), which is allowed to act for one minute, then the slide is again washed with water and dried before examination.

This method was tried out on smears of suspected tuberculous specimens and cultures of acid-fast and nonacid-fast microorganisms (table 1). The technique proved to be of great value in detecting acid-fast microorganisms.

The results of the microscopic examination of 1,210 quadruplet smears from tuberculous specimens showed that 60.1 per cent were positive by the Ziehl-Neelsen method, 60.0 per cent were positive by Kinyoun, 65.1 per cent by Gabbett, and by the new method 65.6 per cent were positive. The mean number of acid-fast bacilli found in each slide per 100 microscopic oil-immersion fields of vision was 524 by the Ziehl-Neelsen method and 961 by the new method (1957).

The staining of 10 different species of acid-fast cultures and 45 different species of nonacid-fast cultures by both methods showed that both techniques did not lose their selectivity for acid-fast organisms.

Trial with duplicate slides from leprous specimens also indicated that our cold-staining method was not inferior to the Kling-Müller method generally used for the staining of *Mycobacterium leprae*. Of 110 leprous smears, 23 were positive and 86 were negative by both methods. One smear was positive by the new method and negative by the Kling-Müller technique. The mean number of leprosy bacilli found in the comparative smears stained by both methods did not vary much.

Preliminary trials also showed favorable results with the staining of acid-fast bacilli in tuberculous tissues. After deparaffinizing, the paraffin sections were stained according to the new method.

In this preliminary examination of 108 duplicate specimens, 70.1 per cent were positive for acid-fast bacteria by the new staining technique and only 52.4 per cent were positive by the Campbell method.

The tubercle bacilli appeared to be more numerous if stained by our method than in similar sections stained by the Campbell technique.

TABLE 1

Number of Specimens	Type of Specimens	Percentage Positive by:					
		Ziehl-Neelsen	Kinyoun	Gabbett	Kling-Müller	Campbell	New Method
1,210	Tuberculous sputum:	60.1	60.0	65.1			65.6
10	Cultures of acid-fast bacteria:	100	100	100			100
45	Cultures of nonacid-fast microorganisms:	0	0	0			0
110	Leprosy specimens:				20.9		21.8
108	Tissue suspected to be tuberculous:					52.4	70.1

KW: TB, dx, lab, microscopy, cold

Gradually more and more laboratories in Indonesia applied this new staining method for the microscopic diagnosis of acid-fast bacilli. By personal communication many investigators expressed the opinion that our method compared favorably with the Ziehl-Neelsen technique.

Four hundred and five medical students who had never had any previous training in acid-fast bacteria staining technique employed the Ziehl-Neelsen and the new staining method in duplicate smears from tuberculous sputum; the impression of 71.3 to 96.9 per cent of the students was that the new method was easier to perform and that the acid-fast bacilli were easier to detect by the new staining technique.

We realize that much more work needs to be done and that our findings require confirmation by other laboratory workers.

Our intention in presenting this report is to stimulate a more thorough investigation of this simple cold-staining technique for acid-fast bacteria, especially for those workers in the tropics where laboratory facilities are often lacking.

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PROPOSAL FOR A TIME-RECORDING PILL DISPENSER AS A METHOD FOR STUDYING AND SUPERVISING THE SELF-ADMINISTRATION OF DRUGS

To the Editor of the American Review of Respiratory Diseases:

In the outpatient management of tuberculosis it is of considerable importance to know whether or not a patient has been taking his medication properly. Similarly, in drug trials carried out on an ambulatory basis this information is crucial. It is the purpose of this communication to show that it is possible to roughly determine when medication is removed from a pill dispenser by incorporating into the dispenser a timing mechanism and thus provide reasonably accurate information about what medication the patient has been taking.

The first thing which comes to mind is the wedding of a clock to a pill dispenser. By utilizing a cylindrical block with holes around the periphery for medication with a clock driven cover that has but one hole for removal of medication, a device could be created that would allow the patient to remove his medication only at the proper time. Forgotten pills could not be removed thus creating a record of the degree of regularity of drug administration and also making it difficult for the patient to take more than the prescribed dose at any one time, which might be important in certain situations like the administration of drugs to psychiatric patients. The obvious difficulty with any clock mechanism is that it is quite expensive and subject to mechanical failure. To avoid these difficulties it is thought possible to substitute for the clock photographic film and a minute radioactive source as a crude but adequate method of determining time intervals.

The device proposed, pictured in figure 1, consists of a wooden or plastic block (figure 2) with holes (*A*) for medication arranged in two circles in a staggered setting around the block. In the center of the block there is a cylindrical depression into which can be fitted 16-mm. photographic film (*B*), covered to prevent exposure to light. In use the block has a plastic cover (figure 3) with two holes (*C*) for the removal of medication. The holes in the cover and underlying block are so arranged that medication can be removed from only one of the holes with any single position of the cover. Built into the cover is a spring-lock mechanism (*D*) which together with pegs (*E*) on the block make it impossible to move the cover except in one direction, one notch at a time, and only after the button (*F*) has been pressed. Thus, the patient must press the button (*F*) and move the cover each time he wishes to remove medication. In addition there are pegs (*G*) attached to the cover and block which prevent the cover from being moved more than one full turn without opening the device. Around the periphery of the main block are markings for the days of the week opposite the holes for medication.

Attached to the cover is a source of radioactive material (*H*) so arranged that it exposes different sections of the photographic film (*B*) with each movement of the cover. By experimentation it has been found that approximately 0.1 of a microcurie of chlorine 36 sealed in plastic to a depth of one millimeter will produce the approximate degree of exposure required when exposed to Kodak dental film. The cover is attached to the block by means of a central bar (*I*) and locked in place by the lock mechanism (figure 4). The lock consists of a steel latch (*J*) that can be released only by use of a moderately strong magnet brought up to the side of the lock. Although pictured here in clear plastic, the final device will probably be made of opaque