# IMPROVED TECHNIQUE FOR THE DETECTION OF ACID-FAST BACILLI BY FLUORESCENCE

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Hagemann (Muench. Med. Wochschr. **85**:1066, 1938) discovered that tubercle bacilli have selective affinity for phenol-auramine stain. Since then, several investigators (Richards, Kline, and Leach, Am. Rev. Tuberc. **44**:255, 1941; Bogen, Am. Rev. Tuberc. **44**:267, 1941; Lempert, Lancet **2**:818, 1944; Gray, Am. Rev. Tuberc. **68**:82, 1953; Kuper and May, J. Pathol. Bacteriol. **79**:59, 1960) have advocated its use in fluorescence microscopy for the demonstration of acid-fast bacilli. However, it has had little acceptance as a clinical laboratory procedure. This may be accounted for by: (i) the danger of false-positive findings due to fluorescing artifacts often present in smear preparations, and (ii) primary and secondary fluorescence of tissue components. This report describes a modified method based on the fact that ferric chloride blocks or reduces most of the interference fluorescences (Clark and Hench, Am. J. Clin. Pathol. **37**:237, 1962).

Two solutions were required: 10% aqueous ferric chloride and phenol-auramine. The stain was made of 3 g of auramine O dye, 40 ml of phenol, and 60 ml of glycerol in 900 ml of distilled water. It was used for staining mycobacteria in body fluids, sputum concentrates, tissue smears, fixed tissues, and culture smears. Smears were fixed in dry heat at 82 to 86 C for 8 to 10 min. Deparaffined tissue sections were brought down to water. Smears and tissue sections were stained for 10 min in phenol-auramine at room temperature and washed well in tap water; ferric chloride was applied for 10 min. Smears and sections were then washed, air-dried, and mounted in a non-fluorescing medium.

A blue-light exciter filter and an orange-yellow barrier filter were used. More fluorescence was achieved with blue than with ultraviolet light. An Osram HBO, 200-w lamp provided adequate illumination for binocular observations. Objectives with good resolving power were required. We used both semiapochromatic objectives and apochromatic objectives with compensating eyepieces.

In smears, contrast between the bacilli and the background was pronounced. Under low magnification, self-luminous, golden-yellow bacilli showed clarity of cell form including beading. They were easily detected in tissues. The yellowish-green color of the sections provided enough detail for histological orientation and made a contrasting setting for the mycobacteria. Cords and microcolonies demonstrated distinct morphology and intense luminosity.

In two sets of experiments, we tested the sensitivity and the specificity of the proposed method (Table 1). In experiment 1, a random group of 400 sputa from tuberculosis wards were processed by both the Kinyoun (Am. J. Public Health **5**:867, 1915) and the phenol-auramine stains. Culture and guinea pig inoculations were made from each specimen. The results of the acid-fast staining were unknown to the observer until the result of the fluorescent stain had been reported. The fluorescent staining yielded 105 positives compared with only 60 by the acid-fast staining. All the 105 fluorescent positives yielded positive cultures, positive guinea pigs, or both.

To confirm or refute this apparent specificity, a second experiment was performed with a view

TABLE 1. *Comparison of the sensitivity and specificity of the proposed fluorescent staining method and Kinyoun's acid-fast staining**

| Expt no. | Stain | Fl+ | Fl− | Total |
|---|---|---|---|---|
| 1 | AF+ | 60 | 0 | 60 |
| | AF− | 45 | 295 | 340 |
| | *Total* | 105 | 295 | 400 |
| 2 | | | | |
| TB wards | AF+ | 37 | 4 | 41 |
| | AF− | 26 | 140 | 166 |
| | *Total* | 63 | 144 | 207 |
| Non-TB wards | AF+ | 2 | 0 | 2 |
| | AF− | 0 | 191 | 191 |
| | *Total* | 2 | 191 | 193 |

* Fl = fluorescent staining; AF = acid-fast staining.

to an increased exposure to the danger of false positives. In this series, sputa from tuberculosis and nontuberculosis wards were used (Table 1, experiment 2). The sequence was randomized according to a table of random digits, leaving the investigators ignorant of the origin of any of the specimens as well as of the results of the acid-fast staining until they had reported their results.

The results of the second series of 400 show, with respect to the 207 specimens from the tuberculosis wards, results in keeping with the results of the first series. The fluorescent staining revealed 63 positives against 41 by the acid-fast staining. However, in four cases the fluorescent stain was reported negative whereas Kinyoun's stain showed acid-fast bacilli.

The results with the specimens from the nontuberculosis wards lend further support to the stipulation of specificity. Of the 193 cases only 2 were positive, both by acid-fast and fluorescent staining. This was the first indication of subsequently confirmed tuberculosis.

In summary, the proposed fluorescent staining method is more sensitive and not less specific than Kinyoun's acid-fast staining.

## DETECTING CELLULOSE-DIGESTING BACTERIA

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A method for distinguishing colonies of certain bacteria which appear to digest carboxymethylcellulose was found during attempts to detect and count cellulase-producing bacteria in the stools of human volunteers before and during the feeding of a diet containing the microcrystalline cellulose Avicel (American Viscose Corp., Philadelphia, Pa.). (This study will be reported by the Metabolic Division, U.S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver, Colo.)

Various media which might indicate colonies of cellulose digesters in plated dilutions were tried without success. These included media containing powdered filter paper or Avicel in agar. When, however, carboxymethylcellulose (carboxymethylcellulose Na, high viscosity type 70; Hercules Powder Co., Wilmington, Del.) was incorporated in agar and poured over an agar medium previously streaked with dilutions of digested sewage sludge, after 4 to 5 days of incubation colonies of certain bacteria were surrounded by depressed areas. Earlier growth was accompanied by small "half-moons" or "breaks" in the medium. The depressions were easily recognizable (Fig. 1), and could be counted readily. These changes were considered to be caused by digestion of carboxymethylcellulose, since they did not occur in agar overlays containing powdered filter paper or Avicel.

This method was applied to dilutions of feces from Avicel-fed volunteers before and after the Avicel diet was begun. An agar medium was prepared containing the following: distilled

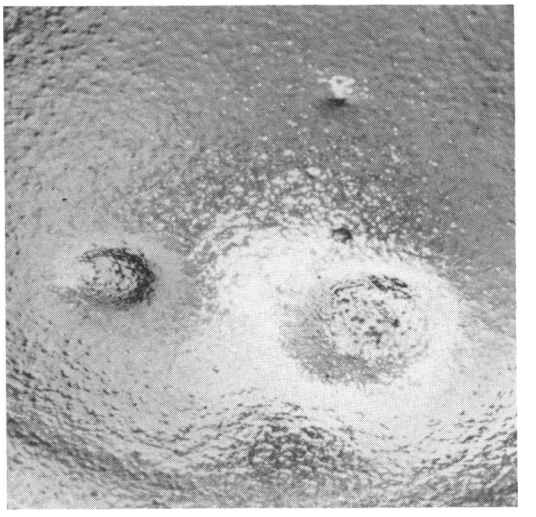

FIG. 1. *Depressed areas surrounding colonies of bacteria on carboxymethylcellulose agar. Magnification:* ×2.