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Evaluation of Frozen Fixed Smears for Use in Fluorescent Antibody Studies of Salmonellae

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Smears of broth cultures of 28 *Salmonella* serotypes were fixed with Kirkpatrick fixative and stored at −20 C. Results indicate that organisms retain the ability to stain at maximal fluorescence intensity for as long as 2 years.

Maintaining standard antigens for evaluating the performance of fluorescent antibody (FA) reagents is often a problem in most laboratories. Bacteria with multiple antigens, such as salmonellae, may vary in the proportion of their antigenic components from one transfer to the next. This results in varying fluorescent intensities which make evaluation of a conjugate difficult.

The following procedure was developed to alleviate this problem with *Salmonella* strains representing O groups A through S, which are needed to evaluate polyvalent *Salmonella* OH conjugates.

Salmonella stock cultures were maintained at room temperature on a stab medium recommended by the late P. R. Edwards. It consisted of 12 g of nutrient agar (Difco), 4 g of nutrient broth (Difco), and 4 g of sodium chloride. The ingredients were dissolved in 1,000 ml of distilled water, dispensed in 4-ml amounts in tubes (13 × 100 mm), and autoclaved at 121 C for 15 min. The inoculated stabs were sealed with waxed cork stoppers.

Antigens for FA staining were prepared by transferring a loopful of stab culture to 5 ml of Trypticase-soy-tryptose broth (BBL). This medium was prepared by mixing equal parts of Trypticase-soy broth (BBL) and tryptose broth (Difco). No attempt was made to enhance motility. The inoculated broths were incubated at 35 C for 12 to 18 h, and then the cultures were killed by addition of a 37% formaldehyde solution to a final concentration of 0.6%.

A smear of each antigen was made within a well of a Teflon-coated multi-well slide (1). The smears were air-dried and fixed for 3 min in Kirkpatrick fixative (six parts absolute ethanol, three parts chloroform, and one part 37% formaldehyde solution). After fixation, the smears were rinsed with 95% ethanol, allowed to drain dry, and then stained for 30 min at room temperature with polyvalent *Salmonella* OH conjugates of known potency. *Salmonella* strains that exhibited maximal fluorescence of 4+ intensity were selected for storage. Strains exhibiting unsatisfactory fluorescence were discarded, and new strains of the same serotype were obtained. When new strains were not available, it was necessary to streak agar plates and pick numerous colonies to find one with a full complement of O antigens.

Sets of smears of the 28 *Salmonella* serotypes representing O groups A through S were prepared, fixed, and stored in sealed, wooden slide boxes at −20 C. Sets were removed at various intervals, thawed at room temperature, and then rinsed with absolute ethanol to remove moisture. The slides were stained and examined under a fluorescence microscope.

Frozen, stored smears of all of the 28 *Salmonella* serotypes retained their ability to stain at an intensity of 4+ with the working dilution of the polyvalent *Salmonella* conjugates over a period of 2 years.

Smears of 24- and 48-h tetrathionate broth cultures of various food and feed samples containing salmonellae were fixed and stored under the same conditions. These remained stable and exhibited 4+ fluorescence of the salmonellae after 9 months of storage at −20 C.

All of the FA-positive results reported in this study were based on the presence of peripherally stained cells typical of salmonellae regardless of whether the organisms had stained flagella. The presence of FA-stained flagellae attached to fluorescing bacterial cells is additional evidence of the presence of salmonellae, but still constitutes a presumptive report, because many *Arizona* strains possess both somatic and flagellar antigens in common with salmonellae.

For the pure culture studies, the Center for Disease Control conjugate (A through I, K, L,

and O) and Difco, Sylvana, and Clinical Sciences polyvalent (A through S) conjugates were used with equivalent results. Only the three commercial reagents were used for staining smears from foodtuffs.

The availability of control antigen smears has greatly facilitated the testing of *Salmonella* polyvalent reagents by eliminating the time-consuming work-up of stock strains. They also serve as positive control smears when diagnostic specimens are examined. The stability of frozen smears from enrichment broth cultures should prove to be helpful in diagnostic laboratories when delays of several days or more occur before examinations can be completed. For short periods of time, the fixed smears may be stored in the refrigerator.

LITERATURE CITED

1. Thomason, Berenice M. 1971. Rapid detection of *Salmonella* microcolonies by fluorescent antibody. Appl. Microbiol. **22**:1064-1069.