Evaluation of the Salmonellae Fluoro-Kit for Fluorescent-Antibody Staining

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An evaluation of the newly developed Clinical Sciences, Inc. Salmonellae Fluoro-Kit, which attempts to standardize the various aspects of the fluorescent-antibody (FA) procedure, was performed with 120 naturally contaminated human food, animal feed, and raw material samples. The Association of Official Analytical Chemists (AOAC) method for the detection of salmonellae was used as the control method. The Fluoro-Kit was found to be simple and convenient to use. The results of this preliminary study show an industrially acceptable rate of recovery of salmonellae by using the Fluoro-Kit in comparison with the A.O.A.C. method. The Fluoro-Kit shows promise as a rapid, salmonellae FA screening method. Problems originally encountered in the application of the Fluoro-Kit are discussed. According to the manufacturer, strict adherence to the new revised procedures included in the Fluoro-Kit will control these problems.

The fluorescent-antibody (FA) technique can be of value when used in the rapid detection of salmonellae (1-7, 9-11). The FA method shortens the detection procedure, thereby producing an elapsed time reduction of several days with a significant cost saving.

The basic unit of the FA method is the slide preparation and staining procedure. For the first time, a commercially available Salmonellae Fluoro-Kit has been developed by Clinical Sciences, Inc. (CS1), Whippany, N. J., which attempts to standardize the various aspects of the FA staining procedure.

This study was performed to assess the accuracy, convenience, and adaptability of the Fluoro-Kit when applied to naturally contaminated food, feed, and raw material samples.

MATERIALS AND METHODS

Samples. In this study, 120 naturally contaminated human food, animal feed, and raw material samples were screened. The sample types tested were: banana crystals, bone meals, frozen chicken, chocolate chunks, chocolate powder, egg albumins, egg noodles, frozen whole eggs, frozen frog legs, meat meals, fish meals, nonfat dried milk, rice product, soya flour, lactalbumin, soya meal, and a wheat product. These samples had been supplied to the investigators through the Food and Drug Administration and other sources.

Cultural method. A 25-g sample was pre-enriched in 225 ml of FA salmonellae broth (Difco). Blending of some samples in a Waring blender for 1 min at high speed was performed to obtain a homogeneous suspension. The samples in pre-enrichment broth were incubated for 18 to 24 hr at 35 ± 2 C.

After incubation, 2 ml was withdrawn from the top third of the pre-enrichment broth and transferred to 18 ml of selenite-cystine broth (Difco). This selective enrichment broth was incubated for 18 to 24 hours at 35 ± 2 C. The slides for 60 of the samples were prepared directly from the selective enrichment broths.

For the second group of 60 samples, 2 ml was withdrawn from their selenite-cystine selective enrichment broths, transferred to 18 ml of FA salmonellae broth, and incubated for 5 hr at 35 ± 2 C. Slides were then prepared in complete accordance with the manufacturer's printed instructions.

Cultural confirmation. After incubation of all selenite-cystine broths, a cultural confirmation procedure was performed by streaking 3-mm loopfuls of the culture on Brilliant Green (Difco), salmonella-shigella (Difco), and bismuth sulfite (Difco) agar plates for simultaneous evaluation by the Association of Official Analytical Chemists method (6).

Fluoro-Kit. The Clinical Sciences, Inc. Salmonellae Fluoro-Kits (supplied by Scientific Specialties, Ltd., Garden City, N. Y.) consist of ten Clinical Slides, each containing 10 wells coated with a biological adhesive (designed to effect maximum sample retention), one reagent control slide with unstained salmonellae organisms, one fluorescent intensity reference slide with stained salmonellae organisms, 1 ml of polyvalent lyopholized salmonellae "OH"
globulin (reported to cover somatic groups A through S) with a dispensing cartridge, phosphate-buffered saline (PBS) crystals, CSI fixative, CSI fixative rinse, CSI mounting media, and CSI cover slips.

Staining method. For each sample tested, a 1.45-mm loopful of culture was applied to one of the coated wells of the Clinii-Slide. The slide was allowed to air-dry at room temperature on a flat surface. The slide was then flooded with CSI fixative and covered for 3 min with a petri dish to minimize evaporation. The slide was then rinsed with the CSI fixative rinse and allowed to air-dry. One drop of the conjugated reagent was placed on each well and allowed to stain for 30 min in a moist chamber at room temperature. The slide was then gently rinsed with PBS followed by two 5-min soaks in PBS and rinsed gently with distilled water. After air-drying, a cover slip was placed on the slide with one or two drops of the CSI mounting fluid.

All slides were viewed on a Wild Heerburg microscope equipped with an Osram HBO-200 mercury arc burner, ×50 and ×100 immersion fluorite objectives; a BG-38 heat-absorbing filter; a BG-12 blue exciting filter transmitting at approximately 425 nm; a OG-1 blue absorbing eyepiece filter; a darkfield condenser, and ×15 wide-angle eyepieces. Darkfield microscopy was utilized to distinguish bacteria from debris.

The criteria for a positive reaction, as viewed on a prepared microscopy slide were: (i) typical salmonellae morphology with or without attached flagella under darkfield and ultraviolet (UV) light and (ii) cells yielding a 3+ or 4+ degree of fluorescence under UV light (12).

RESULTS

One-hundred seventeen (97.5%) of the 120 samples stained with the Fluoro-Kit yielded agreement with the cultural confirmation procedure for salmonellae (Tables I and II). However, a mechanical problem in which salmonellae cells from positive samples on a slide were washed onto the negative samples, thereby making a negative sample appear positive, was encountered with 14 of these samples. These early "false positives" were eliminated when negative samples were isolated and placed on a separate slide.

Three real "false positives" were encountered while using the Fluoro-Kit. Two were the result of the nonspecific staining of a strain of Escherichia coli. The third false positive was produced by an unidentified, nonsalmonella organism. No "false negatives," i.e., slide negative-cultural positives were observed in this study.

Occasionally, while reading the slides, organisms other than salmonellae were observed to be fluorescing. Generally, their morphology was not that of salmonellae; however, their presence was distracting and could cause some difficulty in interpreting the slides. In two instances, smears contained flagellated cells whose flagella, but not the cell wall, were

| TABLE 1. Results for the individual test samples |
|-----------------------------------------------|
| Samples                      | Agreement | False positives |
|-------------------------------|-----------|-----------------|
| Banana crystals               | 2         |                 |
| Bone meals                    | 4         | 2               |
| Frozen chicken                | 1         | 1               |
| Chocolate chunks              | 2         |                 |
| Chocolate powder              | 4         |                 |
| Egg albumins                  | 5         | 3               |
| Egg noodles                   | 2         |                 |
| Frozen whole eggs             | 2         | 2               |
| Fish meals                    | 6         | 2               |
| Nonfat dried milk             | 3         | 1               |
| Rice product                  | 2         | 1               |
| Soya flour                    | 2         | 1               |
| Soya meal                     | 2         | 2               |
| Wheat product                 | 2         | 2               |
| Frozen frog legs              | 2         |                 |
| Lactalbumin                   | 4         |                 |
| Meat meals                    | 13        | 40              |
| Totals                        | 26        | 77              |

| TABLE 2. Slide results and cultural confirmations (CC) for samples tested |
|-----------------------------------------------|
| Agreement | False positives | False negatives |
|-----------|-----------------|-----------------|
| Slide+, CC+ | 26 = 21.66%   | Slide+, Slide rerun++, CC- | 3 = 2.5% |
| Slide-, CC- | 77 = 64.16%   | Slide+, Slide rerun++, CC- |่อย-0 |
| Slide+, Slide rerun-, CC- | 14 = 11.66% | Slide+, CC+ | 0 = 0% |
| Total     | 97.5%          | 2.5%            | 0%            |
fluorescing. These were not identifiable as salmonellae. In some samples, the level of background fluorescence was high and hampered the slide reading process.

The 60 samples which had received the additional 5-hr elective enrichment generally yielded slides with less sample debris, lower background fluorescence, and brighter, fluorescing salmonellae cells. These factors can be a decided advantage in facilitating and shortening time required in observing slides for fluorescing cells. This was the object of this minor modification of the method.

DISCUSSION

A commercially available FA staining system such as the CSI Salmonellae Fluoro-Kit should be very helpful to industrial and commercial laboratories. It eliminates time-consuming preparations while standardizing all aspects of a staining procedure.

The Fluoro-Kit was considered to be simple and convenient to use. It was possible to place as many as 10 samples on one slide, thereby greatly reducing scanning time at the microscope. The conjugated salmonella reagent supplied with the Fluoro-Kit was considered satisfactory. However, with the volume of antisera supplied, it was possible to test approximately 75 samples per kit, rather than the 100 suggested by the manufacturer.

The major problem encountered while testing the Kit was the washing of the salmonellae cells from one slide well to another, producing a number of early false positive results. In those cases in which this problem arises, its effects can be minimized by separating the suspect samples onto individual slides.

The manufacturer has been informed of our test results and suggests that this problem be corrected by strict adherence to the manufacturer’s revised suggested procedure supplied in a new package insert originally not available in the earlier production lots of the Fluoro-Kit.

In this study, the Fluoro-Kit produced a very low (2.5) percentage of true false positives. Industry retains the option to decide what level of false positives can be tolerated and still retain an economic and time advantage by using the FA method.

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