The Food and Drug Administration (FDA) has primary responsibility in the federal government for food safety in the United States. FDA is charged with the enforcement of the Federal Food, Drug, and Cosmetic Act (FFDCA), and food safety related aspects of the Public Health Service Act (PHSA). The FDA mandate under these Acts includes widespread responsibilities to help ensure preharvest food safety. One mission of FDA’s Center for Veterinary Medicine (CVM) is to regulate the levels of contaminants permitted in animal feeds to ensure that the food for man and animals is safe and free of illegal drugs, industrial chemical, pesticide residues, and harmful bacteria. To meet this mandate, CVM has emphasized the application of HACCP programs in the feed industry as an approach for achieving salmonella negative feed. To establish a baseline in the feed industry, CVM has conducted surveys to determine the prevalence of salmonella contamination in animal and vegetable proteins, complete feed, and the primary protein meal ingredient.

Complete feed and primary meal ingredient samples were collected from commercial feed mills and on-farm mixers. The on-farm mixers were randomly selected from FDA’s Official Establishment Inventory (OEI). Each district was assigned a specific number of commercial feed mills based on the number of registered feed mills within the district. The district was instructed to select mills that would be representative of the feeds manufactured in the district. Medicated feeds were included in the sampling. Each sample of meal and complete feed consisted of 30 individual subsamples which were aseptically collected. Analysis followed the procedures outlined in FDA’s BAM and included serogrouping and serotyping. The results being reported today represent data from 1,980 meal subsamples and 1,860 complete feed subsamples covering 66 meals and 62 complete feeds respectively.

Sixteen percent of the complete feeds and 48% of the meals were positive for Salmonella. When the meals were grouped by animal and vegetable source, 82% of the animal meals and 37% of the vegetable meals were positive for Salmonella. When the meal and complete feed pairs were compared, if the meal was positive, the complete feed was also positive in 30% of the samples. There was one instance in which the meal was negative and the complete feed positive (3%) and 32 instances in which both the meal and feed were negative (97%).

A more complete text can be located after the breakout group reports.

The following is a summary of the research conducted by Dr. Amy Waldroup and her co-workers at the University of Arkansas under an FDA contract. The research was to optimized the culture conditions for detecting Salmonella in a feed matrix and to evaluate several rapid detection kits for their ability to detect Salmonella in a feed matrix. The rapid detection kits were used in accordance with the manufacturers directions. This summary incorporates Dr. Waldroup’s comments and was prepared by Dr. Dan McChesney of FDA / CVM.

**SUMMARY**

Results obtained from these studies suggest that the culture method described by Bailey and Cox (1992) for the simultaneous recovery of Salmonella and Listeria from food samples can also be used for detection of Salmonella in feed ingredients and finished feeds. This culture method, which includes preenrichment in Universal Preenrichment broth, is capable of detecting as few as 1 to 2 salmonellae
(either nalidixic acid-resistant or indigenous species) in 100 g of finished feed or animal byproduct meal. It should be noted that in all of the present studies sample size was 100 g due to the extremely low level of salmonellae typically reported for feeds and feed ingredients. Universal preenrichment broth was superior to lactose broth for the detection of indigenous salmonellae in meat and bone meal, regardless of assay procedure.

In an interlaboratory study involving five laboratories, the Bailey and Cox (1992) culture procedure resulted in 1.3% false negative feed or feed ingredient samples and no false positive samples. The SAL-TEK procedure resulted in 3.1% false negative samples, and 6.2% false positive samples. However, half of the false positive samples and 3 of 7 of the false negative samples which occurred when the SAL-TEK procedure was utilized were from a single laboratory. Thus, if only the data from the other four laboratories is considered, the culture method of Bailey and Cox (1992) and the SAL-TEK procedure have almost identical false negative and false positive frequency rates.

Based on these studies, the contractor suggested a minimum sample size for finished feeds or feed ingredients of 100 g, preenrichment in Universal Preenrichment broth, followed by either a standard culture procedure or the SAL-TEK immunoassay.

In addition to the SAL-TEK, the GENE-TRAK, CAP-TEK, TECRA UNIQUE, and direct plating of Salmonella on immunobeads were evaluated for the ability to detect Salmonella in naturally contaminated feed samples.

The GENE-TRAK procedure resulted in no false positive samples, but did result in 10.3% false negative samples. These false negative samples were spread out among various inoculum levels and even included samples to which salmonellae were added at the rate of 140 CFU/100 g feed.

CAP-TEK procedure resulted in a higher number of false positive and false negative results than with SAL-TEK or the culture method. For samples with very low levels of, the elimination of the selective enrichment step (as in the CAP-TEK method) may not be advisable.

The TECRA UNIQUE method produced an unacceptable number of false negative samples. The number of Salmonella present in the preenrichment culture was postulated by the author to not to have been high enough for detection by the TECRA UNIQUE method. Earlier preliminary tests utilizing TECRA UNIQUE for feeds with known levels of inoculated Salmonella had shown it to be comparable to the culture method.

The direct plating of Salmonella on immunobeads also produced too many false negative results. A potential problem noted with this method was that it was extremely labor intensive. This method did, however, reduce detection time and media needed for samples that contain moderate levels of Salmonella.

In summary, a series of studies utilizing naturally contaminated feed ingredients or finished feeds obtained from FDA, suggested that for feed ingredients or finished feeds either the SAL-TEK or the culture method of Bailey and Cox (1992) will provide the most accurate and consistent results. Because of the low levels of Salmonella in the naturally contaminated samples tested, extreme reductions in the total time allowed for growth may not be advisable and could lead to an unacceptable number of false negative samples.

Finally, it was noted by Dr. Waldroup that it would be very difficult to perform either the culture method or the SAL-TEK at a facility that did not have any standard microbiological equipment. The typical feed mill will probably not have the required facilities or equipment for either the culture method or a rapid kit.
Culture Method Utilizing Universal Preenrichment Broth (UPB)
Bailey and Cox, 1992

1. Add 100 g of feed sample to 900 ml of UPB by sprinkling feed onto the surface of UPB. Allow the feed to soak undisturbed for 30 minutes. Mix well, loosen caps if bottle is used and incubate for 18 to 20 hours at 35 C. Because of the large quantity of UPB it is advisable to prewarm the liquid to 30 to 35 C prior to adding sample. Alternately, large freezer ziploc bags can be used to hold sample and UPB if these bags can be held upright in incubator.

2. Swirl the sample gently, allow loose particles to settle and transfer 1.0 ml of incubated broth to tubes of tetrathionate-Hajna broth (10 ml/tube) and 1.0 ml to selenite cystine (10 ml/tube). Incubate tetrathionate-Hajna tube at 42 C for 20 to 24 hours. Incubate selenite cystine tube at 35 C for 20 to 24 hours.

3. Streak incubated tetrathionate-Hajna and selenite cystine broths onto separate brilliant green sulfa, modified lysine iron agar, and XLT-4 agar plates. It is convenient to use tri-plates for these three selective agars. Incubate plates for 24 to 26 hours at 35 C.

4. Pick, stab, and streak typical colonies onto triple sugar iron and lysine iron agar slants. Loosen caps and incubate for 24 hours at 35 C.

5. Serologically screen with poly 0 somatic antigen using the slide technique.

6. For atypical colonies, biochemically characterize isolates with conventional methods or API or Micro-ID miniaturized kits.

7. Serologically confirm using poly H flagellar antigens. Use the tube technique.