CHAPTER FIVE

Detection Methodologies for Microbes and Chemicals

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The safety of the food supply is a significant issue for many democratic nations today. In recent years, public health concern has shifted from residues of animal drugs, pesticides, and environmental contaminants to microbiological issues involving human pathogens and their association with illness and mortality. Consequently, methods and technologies for detecting pathogenic microorganisms have become central to efforts to ensure a safe food supply. Even for residues, where animal drugs are the key concern, microbiological tests based on a residue inhibiting a prepared microbiological culture are crucially important. Today I will describe the need for the Food Safety and Inspection Service (FSIS) to develop or procure detection methods for microbes and chemicals that will meet its regulatory responsibilities.

FSIS has available four distinct approaches to method development and procurement: Agency evaluation of commercially-available test kits for use in FSIS food-testing laboratories; AOAC or other third-patty verification of commercial methods; internal FSIS development or modification of methods at the FSIS laboratory in Beltsville, Maryland; and contracting for methods development. In what follows I will describe the specific needs the Agency sees as central to its mission of protecting the public health from foodborne hazards.

RESIDUES

The FSIS National Residue Program comprises more than ten classes of compounds and some 75 individual compounds, and thus requires a wide variety of analytical and microbiological methods. Screening tests, primarily microbiological, are used in-plant or in the laboratory. Complementing these screening tests are quantitative laboratory methods using different extraction, isolation, purification, concentration, and detection procedures. Rigorous confirmation of a residue, when possible, is done with methods that identify and/or verify the structure of the analyte.

Chlorinated hydrocarbon pesticides and a number of chlorinated organophosphate pesticides leave residues in fat that can be extracted by either adsorption or gel permeation chromatography (CPC). Residues from non-chlorinated organophosphates are found in liver tissue; organophosphates metabolize quickly, with few exceptions and confirmation of these residues is difficult to accomplish. Other pesticides commonly included in the National Residue Program are carbamates (extracted from liver tissue, followed by CPC);chlorinated triazines (extracted from fat, followed by capillary gas chromatography); and pyrethroids (extracted from fat, followed by gas chromatography). Enzyme-linked immunosorbent assays, or ELISA's have been developed that can detect some of the pyrethroids, but cannot identify or quantitate individual compounds.

As was mentioned above, the primary residue concern is with animal drugs. Some analytical methods developed by manufacturers of animal drugs—as is required for approval—may be too resource-intensive when many samples must be processed rapidly, and there is a general need for better analytical methods that are reliable,

rugged, and accurate.

Residues from antibiotics and sulibnamides are screened for by microbial inhibition assays, the most common being the Swab Test on Premises (STOP), used with many species, and the Calf Antibiotic and Sulfonamide Test (CAST). The FSIS Microbiology Division has recently developed a new test for antibiotics and sulfonamides in calves and cows, the Fast Antimicrobial Screen Test. FAST is showing that it can produce reliable results sooner than CAST or STOP.

Positive in-plant STOP results are verified through laboratory analysis with the same test used in-plant. The basic procedures then employed for determining antibiotic residues are bioassays using test organisms for various antimicrobials. Through the use of standard inhibition patterns, fingerprint profiles for the classes of antimicrobials have been developed. These microbial inhibition bioassays are useful because they detect almost all members of several antibiotic classes at relatively low cost, but they are not exhaustive.

Chemical antibiotic methods are in limited use, and the Food and Drug Administration (FDA) is currently encouraging development of chemical methods for supplementary quantitation and confirmation of antibiotics. In some cases, such as apramycin, FSIS combines microbial inhibition assays with thin layer chromatography.

Typical of the lack of analytical chemistry methods for antibiotics are the aminoglycosides—such as neomycin, gentamicin, and streptomycin—for which chemical analysis is difficult and there are no validated analytical methods. Macrolide antibiotics—such as erythromycin, novobiocin, and tylosin—are a similar case in that effective chemical methods for their detection are limited and are usually only confirmatory. Tetracyclines also present difficulties, and there are few analytical methods available.

Suitable analytical methods have been successfully developed and have been used for the anabolic hormones DES and zeranol, and for the progestational agent MGA. Such is not the case with the principal class of anthelmintics—the benzimidazoles. The methods developed by manufacturers for these drugs are compound-specific and do not lend themselves to a multi-residue analysis. Adequate methods do exist for other anthelmintics in limited use—morantel and pyrantel tartrate, and dibutyltin dilaurate. An important class of compounds comprises the coccidiostats dimetridazole, ipronidazole, and ronidazole. The total residue picture for these compounds is ambiguous, and this fact, coupled with their toxic and carcinogenic potential, has led to the introduction of other, alternative compounds. Among these are the ionophores—such as monensin and lasalocid. The ionophores are themselves difficult to detect, and, furthermore, they tend to metabolize rapidly with only a small portion of the total residues being the parent compound.

Sulfonamides are much used animal drugs and are significant sources of residues as well. To meet a problem with sulfamethazine residues in swine, FSIS developed a thin layer chromatographic semi-quantitative screening procedure that may be used for in-plant testing of pig urine to identify suspect animals. Residue methods for sulfonamides usually involve thin layer, gas liquid, or high performance liquid chromatography for quantitative determination. Confirmatory methods tend to be gas chromatography-mass spectrometry procedures. A multiresidue method for sulfonamides using thin layer chromatography coupled with fluorimetric scanning densitrometry for quantitation has been developed and extended to thirteen sulfonamides with confirmation using gas chromatography-mass spectrometry. Recent approaches have used reverse phase liquid chromatography with ultraviolet detection; the procedure has proved reliable in regulatory laboratories but presents difficulties with solvents.

Perhaps the most widely used veterinary drug in the world is the potent endo- and ecto-parasitic agent ivermectin, which is a mixture of two macrocyclic molecules with little functionality for residue analysis. It must be chemically converted to a fluorescent compound for quantitation and confirmation. The method is labor-intensive, and recent efforts have focused on developing an automated system. Attempts have been made to develop a mass spectrometry confirmation procedure but so far without success.

A class of compounds growing in significance is the beta-agonists, used to increase growth rate and lean/fat ratio. Analytical schemes have been developed for use with animal urine and tissue for residue control programs, and an immunoaffinity chromatography-gas chromatography method has shown efficacy as a multiresidue procedure including clenbuterol, a key public health concern.

This cursory review of a complex residue picture has, I hope, conveyed the many difficult technical problems with residue analysis and control. I will now turn to microbiological methods.

MICROBES

Microorganisms are pervasive in the environment from which we derive our food supply, and in us. While many microbes are harmless to healthy animals and humans, many are less benign and can cause disease and even death. From farm to transport to slaughterhouse, there is a plethora of opportunities for animals to become contaminated with pathogenic microbes. Contamination on meat surfaces arising from fecal contamination at the time of slaughter and dressing can occur randomly in "hot spots" on a carcass. Since most contamination is due to cross-contamination, basic microbial load is usually low. In food microbiology, how-

ever, the presence of a single pathogenic organism is considered significant since not only can infectious dose be low for certain organisms but improper cooling and refrigeration can cause multiplication. Given the variable incidence of pathogen contamination, the irregular "hot spots" on a carcass, the low numbers of pathogens, and the presence of numerous competitive microbes, meat microbiologists face a daunting task.

A key factor in testing for pathogens is the necessity of enriching samples to boost bacterial numbers to levels capable of being detected by standard microbiological methods. This involves a host of considerations: nature of the target organism, sample type, condition of the animal sampled, expected levels of the target organism and possible competitors, competitiveness of the target organism versus other microflora, susceptibility and resistance of the target organism and competitors to antibiotics and microbial inhibitors, growth requirements of the target organism, etc. After a suitable recovery system has been defined, a scheme for confirming the identity of the suspected pathogen must be developed. All current culture-based methods have limitations in sensitivity or specificity. Time, both for development and practice, is the main requirement and challenge for meat microbiology.

Rapid methods have been developed to address the problematics of time, sensitivity, and specificity.

ELISA methods have been used as screens for the detection of pathogens in meat and poultry products. ELISA methods use an antibody specific to a particular pathogen and usually show a colorimetric reaction. Multiple samples can be analyzed simultaneously, the procedure is semi-automated, and the assay takes less than three hours to complete. Some newer ELISA's take 15-30 minutes to perform. A key disadvantage is that ELISA methods are not very sensitive, and loads of greater than 100,000 cells are necessary for a positive reaction. The consequent need for an enrichment step lengthens analysis time to between 27 and 50 hours.

DNA probe tests recognize and hybridize to a characteristic segment of the target organism's nucleic acid. Some probes can be specific for the detection of genus, species, and sometimes strain. DNA probes are being evaluated for *Listeria* and *Campylobacter* and are being sought for *Staphylococcus C. perfringens*, and *E. coli* 0157:H7. DNA probes have certain limitations: large numbers of microbes are required—thus enrichment again; greater technical difficulty and expense than ELISA's; some types of meat samples contain DNA inhibitors that may interfere with some newer molecular technologies; and DNA probes are radioactively labeled, thus requiring appropriate containment and disposal controls in the laboratory.

Polymerase chain reaction technology is emerging as a possible means of detecting pathogens in foods. This technique has the potential of simplifying the detection of low numbers or amounts of analyte, but to date there are few reports of its use to detect foodborne pathogens in routine investigations. It also cannot distinguish between live and dead cells, and greater technical expertise is required to perform the analysis.

An immunocapture antibody-coated bead method developed by FSIS for *E. coli* 0157:H7 can reduce the enrichment time required by most culture methods.

An intriguing possibility is biosensors—devices that would probe into a meat sample directly detect bacterial pathogens, and give an interpretable electrical reading. Basic research on their use for meat and poultry products has yet to begin, and applicable technology will probably be a phenomenon of the 21st century.

In what I have said about microbiological methods, you can see some of the constant issues and challenges. USDA is searching for new methods for the detection of microbes of public health concern and microbial toxins and mycotoxins in raw and ready-to-eat meat and poultry products. We are emphasizing the development of technologies that can detect and enumerate low numbers and decrease costs and turnaround times, and are easier to use so that in-plant testing could become a reality. As I stressed earlier, this latter objective is particularly challenging given the paramount concern with not creating a biohazard in the plant environment.

A potentially relevant FSIS initiative is the Pathogen Reduction/Hazard Analysis and Critical Control Point (HACCP) regulation proposed by the Agency in February 1995. This regulation will mandate industry HACCP programs within three years of becoming final, and rapid in-plant testing for residues and microbes would likely be crucial to such HACCP Systems.