Calf Antibiotic and Sulfonamide Test (CAST) for Screening Antibiotic and Sulfonamide Residues in Calf Carcasses

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The Calf Antibiotic and Sulfonamide Test (CAST), a microbial inhibition screening test, was developed for detecting antibiotics and sulfonamides in bob veal calf carcasses. The test uses *Bacillus megaterium* ATCC 9885 as the indicator organism and Mueller Hinton agar as the growth medium. Compared to Swab Test on Premises (STOP), developed in 1970, this screening test has higher sensitivity and the ability to detect a wider range of veterinary antimicrobial residual drugs, particularly sulfonamides, at lower concentrations. Carcasses that are tested with CAST and suspected of containing chemical residue above tolerance level are retained for confirmation. Disposition of these carcasses are determined upon laboratory result. Routine testing of bob veal calves with CAST allowed the Food Safety and Inspection Service to release most calf carcasses within 24 h post-slaughter, thus conserving shipping and handling resources. However, changes in the regulation in 1990 dictate that disposition of carcasses found to contain violative levels of sulfonamide residues should be based on laboratory findings. The analysis of the data for the years 1990–1994 and 1998 indicate that the use of CAST over the years was significant, and had a direct impact on reduction of residue violations in veal carcasses. With the use of CAST, potentially harmful antimicrobial chemicals entering the human food chain through veal meat have been minimized.

The U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) is responsible for ensuring that the nation’s meat food products are wholesome and unadulterated. Since 1967, FSIS has routinely analyzed a sufficient number of meat and poultry samples to prevent agriculture residues from getting into the human food chain through food animals. In order to meet the demand for analyzing an increasing number of samples every year for various types of residual chemicals, FSIS has introduced new and efficient rapid tests. FSIS implemented its first rapid screen test known as Swab Test On Premises (STOP) in slaughter establishments in 1979. The test was developed in the FSIS Beltsville Methods Development Laboratory (Beltsville, MD; present address: USDA, FSIS, OPHS, Laboratory QA/QC Division, Russell Research Center, Suite 205, 950 College Station Rd, PO Box 6085, Athens, GA 20604) for the detection and control of antibiotic residues in food animal carcasses (1). The test was designed to screen carcasses for the presence of veterinary antimicrobial residues, especially antibiotic residues. This test allowed FSIS inspectors to release residue-free carcasses in 24 h. Prior to the use of STOP, carcasses would have been held for 5–6 days (as were carcasses with violative residues) until the receipt of a laboratory report.

In the 1980s, an increase in the violation rate for sulfonamide and antibiotic residues in veal calf carcasses was noted by the FSIS National Residue Program (2). The incidence of sulfonamide residue violations in bob veal carcasses (calves <3 weeks of age or 150 pounds in weight) rose sharply in 1981 and continued through 1982 (3, 4). The problem appeared to be widespread throughout the Northwest, Mid-Atlantic, and upper Midwest. To ensure the safety of meat and other food products, in 1984, the U.S. Food and Drug Administration (FDA, under section 512 of the Federal Food, Drug, and Cosmetic Act [21 USC 360 (b)], set a
tolerance level for sulfamethazine and sulfathiazole in cattle and swine tissues at 0.1 parts per million (ppm). Subsequently, the evidence of greenish discoloration of the stomachs of slaughtered bob veal carcasses, aptly called “green gut” carcasses, alerted the USDA inspector to sample them for possible drug sulfonamides. In order to screen such animals, the Calf Antibiotic and Sulfonamide Test (CAST) was also developed at the Beltsville Laboratory. To reduce public health risks from veal meat containing antimicrobial residues, the FSIS promulgated an inspection regulation stipulating that any veal calf suspected of suffering from gastrointestinal disorder, including those presenting a “green gut” lesion (5), should be tested at the slaughter establishment using CAST (6). In 1985, the test was introduced in slaughter establishments for screening bob veal calf carcasses for antibiotic and sulfonamide residues (7). Based on the information that changes have occurred in trading and treatment practices in veal calves since the implementation of the residue testing program, FSIS modified the testing procedure in 1987 for detecting violative levels of sulfonamide and antibiotics in veal calves. The modification established a testing rate for an establishment, allowed establishment personnel to perform the test, discontinued testing of animals with pathological condition, and finally authorized the inspecting veterinarian to reduce line speed to allow sufficient time for performing the test (8).

The sensitivity of CAST for detection of various antibiotics and sulfonamides was validated from comparative studies with the Fast Antimicrobial Screen Test (FAST), also developed in the Beltsville Laboratory in 1994 (9). The in vivo and the in vitro comparative studies were conducted in Beltsville and in several slaughter plants in different locations in the United States (10). Since FAST was introduced in the field in 1995, the number of uses of CAST decreased from 204 222 in 1987 to 8444 in 2000 (11, 12). Coincident with use of CAST, residue violation data indicated a substantial decrease in residue violations during the period of its use. The use of CAST likely played an important role in this reduction (13, 14).

Aside from its method of preparation and quality control (15) and an instruction booklet for the inspectors in the field on how to use CAST (7), its efficacy was never published. The test is commercially available in the United States and in some European countries, and FSIS receives regular inquiries on its performance. Although CAST has a wide range of sensitivity for antibiotics and some sulfonamides, its lower limit of detection (LOD) for sulfonamides is above the tolerance level of sulfonamides in animal tissue (16). Thus, in order to improve the detection capability of sulfonamide residues in bovine tissue, FAST was developed and tested under field conditions (10). The sensitivity and specificity of CAST compared to FAST for antibiotics were not significantly different; however, the lower LOD for FAST against sulfonamides was 0.5 ppm, which was 4 times lower than CAST (16). Considering the in vivo and in vitro performances of both tests, FAST was found to be more sensitive to sulfonamides present in bovine tissue and, as a result, since 1995 FAST has been used more often than CAST, particularly to test bovine species (17–19).

The present paper reports the development of CAST, its sensitivity and its ability to detect antibiotics and sulfonamide residues in the bob veal calf kidney. Further, the conclusion of the paper justifies the use of FAST, a better screening test which is being used in the FSIS Residue Control Program. The development of FAST is reported elsewhere (16). The report also analyzes the impact of the test on the residue violation rate in bob veal carcasses for a 15-year period, and correlates the rates with laboratory results obtained by the use of a standard 7-plate bioassay and chemical tests (20).

**Experimental**

**Microorganisms**

For the CAST screen, a stock spore suspension of *Bacillus megaterium* ATCC 9885 was prepared according to the method described (15). From the stock, a working solution with 1 × 10^6 spores/mL was prepared in 50% alcohol (15, 21). A 5 mL spore suspension of *B. subtilis* ATCC 6633 in 50% ethanol containing 1 × 10^6 spores/mL used for the STOP screen was supplied by Editek (Burlington, NC).

**Media and Preparation of CAST and STOP Plates**

Mueller Hinton agar used in test plates was prepared according to manufacturer’s directions and distributed in 6 mL aliquots in 60 × 15 mm plates. Commercially prepared STOP plates (Cat. No. 500151) were supplied by Editek.

**Preparation of Antibiotic Standards**

All antibiotics were obtained from Sigma Chemical Co. (St. Louis, MO). The following standards were obtained: Ampicillin (sodium salt, Cat. No. A-0166); amoxicillin (Cat. No. A-8523); cloxacillin (sodium salt, Cat. No. C-9393); penicillin G (potassium salt, Cat. No. P-7794); gentamicin sulfate (Cat. No. G-3632); neomycin sulfate (Cat. No. N-1876); streptomycin sulfate (Cat. No. S-6501); chlorotetracycline hydrochloride (Cat. No. C-4881); oxytetracycline hydrochloride (Cat. No. O-5875); tetracycline hydrochloride (Cat. No. T-3383); bacitracin (zinc salt, Cat. No. B-5150); erythromycin sulfate (Cat. No. E-6376); oleandomycin (phosphate salt, Cat. No. O-6125); tylosin tartrate (Cat. No. T-6134); virginiamycin (Cat. No. V-2753); novobiocin (sodium salt, Cat. No. N-1628); sulfamethazine (SMZ; sodium salt, Cat. No. S-5637); sulfathiazole (sodium salt, Cat. No. S-0127); and sulfadimethoxine (sodium salt, Cat. No. S-7385).

AOAC-recommended buffers were used for preparing standard solutions of each of the test antibiotics (22). Stock solutions (1000 µg/mL) penicillin G, amoxicillin, ampicillin, cloxacillin, and bacitracin were made in pH 6.0 phosphate buffer. Standard concentrations of penicillin G and amoxicillin (0.0125, 0.025, 0.05, 0.1, and 0.2 µg/mL); ampicillin (0.125, 0.25, 0.5, 1.0, and 2.0 µg/mL); cloxacillin (0.25, 0.5, 1.0, 2.0, and 4.0 µg/mL); and bacitracin (0.5, 1.0,
2.0, 4.0, and 8.0 \( \mu g/mL \), also in pH 6.0 phosphate buffer, were prepared from the stock solution. Similarly, standard concentrations of 0.125, 0.25, 0.5, 1.0, and 2.0 \( \mu g/mL \) gentamicin sulfate, neomycin sulfate, and streptomycin sulfate (0.125, 0.25, 0.5, 1.0, and 2.0 \( \mu g/mL \)) were prepared from the stock solution in pH 8.0 phosphate buffer. Working standards of 0.125, 0.25, 0.5, 1.0, and 2.0 \( \mu g/mL \) concentrations of erythromycin sulfate and oleandomycin were prepared in pH 8.0 phosphate buffer from stock solutions (1000 \( \mu g/mL \)) which were made first by dissolving the salt in 2 mL methanol and then diluting with pH 8.0 phosphate buffer. Working standards of 0.25, 0.5, 1.0, 2.0, and 4.0 \( \mu g/mL \) novobiocin (1.25, 2.5, 5.0, 10, and 20 \( \mu g/mL \)), and virginiamycin (1.25, 2.50, 5.0, 10.0, and 20.0 \( \mu g/mL \)) were also made from stock solutions (1000 \( \mu g/mL \)). Tetracycline, oxytetracycline, and chlortetracycline standards of 0.125, 0.25, 0.5, 1.0, and 2.0 \( \mu g/mL \) were made from stock solutions (1000 \( \mu g/mL \)) which were made by dissolving the salt in 2 mL 1N HCl and diluting with pH 4.5 phosphate buffer to a final concentration of 1000 \( \mu g/mL \). Commercially available 5 \( \mu g \) neomycin susceptibility discs (Difco Laboratories, Detroit, MI) were used to check the sensitivity of test organisms to antibiotics and for quality control of each batch of plates used over the entire study period.

### Source and Preparation of Swabs with Antibiotic Standards

To ascertain the uniformity of liquid samples absorbed by the swabs used in this screen test, 100 commercially available sterile swabs (Fisher Scientific, Fairlawn, NJ, Cat. No. 14-959-91) kept in a desiccator, were weighed and then dipped in sterile distilled water. After the excess water from the swabs was removed by touching the swab tips with an absorbent paper, all 100 were again weighed. The average amount absorbed per swab as tested with 100 swabs was 200 \( \mu L \), and the variation in weight gain by swabs was within \( \pm 5.0 \mu L \).

### Preparation of Discs with Sulfonamides

Six solutions of SMZ with various concentrations were prepared in Butterfield’s phosphate buffer in such a way that each 25 \( \mu L \) solution contained 0.125, 0.5, 1.0, 2.0, 4.0, and 8.0 \( \mu g \) SMZ. A 25 \( \mu L \) aliquot of SMZ solution of one strength was pipetted to each 1/4 in. analytical paper disc (Schleicher & Schuell, Keene, NH; No. 740-E). In this way, solutions of SMZ of the various strengths listed were pipetted to other sets of discs. The discs were dried for 30 min and stored at –15°C. Under these conditions, the antimicrobial activity of the discs against *B. subtilis* remained unchanged for 3 weeks. However, every second week, fresh discs were prepared. As for SMZ, discs containing various concentrations of sulfathiazole and sulfadimethoxine were prepared and stored.

### Optimum Temperature for SMZ (Sulfonamide) Activity

The CAST plates were streaked with \( 1 \times 10^6 \) spores/mL *B. megaterium* in a 5-way pattern as shown in Figure 1, using sterile swabs to ensure an agar surface with uniform distribution of spores. SMZ discs of each concentration were placed on each plate in triplicate, and the plates were incubated at different temperatures to determine the optimum temperature for SMZ activity.

### Table 1. Effect of Incubation Temperature on the Antimicrobial Activity of Sulfamethazine

<table>
<thead>
<tr>
<th>Conc of sulfamethazine in disc, ( \mu g )</th>
<th>Zone diameters, mm at °C*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>29°C</td>
</tr>
<tr>
<td>0.125</td>
<td>NI</td>
</tr>
<tr>
<td>0.25</td>
<td>NI</td>
</tr>
<tr>
<td>0.5</td>
<td>NI</td>
</tr>
<tr>
<td>1.0</td>
<td>NI</td>
</tr>
<tr>
<td>2.0</td>
<td>9</td>
</tr>
<tr>
<td>4.0</td>
<td>10</td>
</tr>
<tr>
<td>8.0</td>
<td>12</td>
</tr>
</tbody>
</table>

*Disc diameter = 6.35 mm; NI = no inhibition; # = inconclusive growth of organisms.*
incubated at 27°C for 18 h. Similarly, other sets of plates with SMZ concentrations were incubated at 29°C, 32°C, 37°C, 45°C, and 50°C for 18 h and then examined for zones of inhibition around each disc (Table 1).

**Performance of CAST**

The procedure for the CAST is as follows: A sterile cotton swab is inserted into a kidney of a freshly slaughtered calf and the swab is allowed to soak in the kidney fluid for 30 min. While the swab absorbs tissue fluids, a *B. megaterium* spore suspension is applied to a CAST test plate by a sterile swab (Figure 1). The numbers identifying samples are written on the side rim of each plate (Figure 2). After 30 min, the swab is removed from the kidney. Excess fluid on the handle is carefully wiped off with a tissue paper without touching the head of the swab. The handle of the swab is broken off about 1/2–3/4 in. below the base of the saturated cotton tip, and discarded. The swab is then placed on the plate. Another swab from another kidney is placed on the same plate. A standard neomycin 5 µg disc is also placed on the lower third of the plate (Figure 2) and the plate is incubated at 45°C. After 16–20 h incubation, swabs are removed from the plate and the zone of inhibition (ZI) around each swab is measured vertically and horizontally and recorded. In order for a tissue to be identified as positive by CAST, a criterion of an 18 mm (horizontal plus vertical measurement of inhibition around a swab) or greater ZI was established.

**In Vitro Evaluation of CAST**

STOP and CAST plates were prepared by streaking STOP plates with 1 × 10⁶ spores/mL *B. subtilis* and CAST plates with 1 × 10⁶ spores/mL *B. megaterium*. Cotton swabs containing various sulfonamide and antibiotic solutions were placed on STOP and CAST plates. A neomycin disc (N5 µg) was placed on each plate. The STOP plates were incubated at

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>Antimicrobial concentration, ppm/Zone of inhibition, mm</th>
<th>Tolerance in kidney, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin</td>
<td>0.5/9.0</td>
<td>0.25/9.0</td>
</tr>
<tr>
<td>Neomycin</td>
<td>1.0/8.0</td>
<td>0.25/11.0</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>0.5/9.0</td>
<td>0.25/10.0</td>
</tr>
<tr>
<td>Chlortetracycline</td>
<td>0.25/8.0</td>
<td>0.25/11.5</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>3.0/10.0</td>
<td>0.5/10.0</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>2.0/11.0</td>
<td>0.25/11.0</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0.25/9.5</td>
<td>0.1/10.0</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>0.25/8.0</td>
<td>0.05/10.0</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>1.0/9.0</td>
<td>0.5/9.0</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>0.05/9.0</td>
<td>0.01/9.0</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>10.0/NI</td>
<td>10.0/NI</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.25/11.0</td>
<td>0.05/9.0</td>
</tr>
<tr>
<td>Oleandomycin</td>
<td>0.25/10.0</td>
<td>0.25/10.0</td>
</tr>
<tr>
<td>Virginiamycin</td>
<td>10.0/10.0</td>
<td>0.25/10.0</td>
</tr>
<tr>
<td>Novobiocin</td>
<td>20.0/9.0</td>
<td>1.0/9.5</td>
</tr>
<tr>
<td>Sulfamethazine</td>
<td>4.0/NI</td>
<td>1.0/9.5</td>
</tr>
<tr>
<td>Sulfathiazole</td>
<td>4.0/NI</td>
<td>1.0/10.0</td>
</tr>
<tr>
<td>Sulfadimethoxine</td>
<td>4.0/NI</td>
<td>0.25/10.0</td>
</tr>
</tbody>
</table>

* Averages of 3 determinations.
* * = Not approved for cattle; ** = no tolerance in cattle.
* NI = No inhibition.
29°C for 16–20 h, and the CAST plates at 45°C for the same period of time. The average ZI around each swab measured horizontally and vertically was recorded, and the lowest concentration of each antimicrobial which produced a clear ZI of an average of at least 8 mm was noted (Table 2).

In Vivo Evaluation of CAST

From 1990 to 1994, calf kidneys presenting a ZI of 18 mm or greater at slaughter establishments using the CAST test and the corresponding liver and muscle tissue from those carcasses were analyzed by a 7-plate bioassay for the presence of antibiotics at the FSIS laboratory in Beltsville. The samples which were suspected to contain sulfonamide drug were then analyzed chemically (20). To determine if the performance of CAST had changed significantly over the years, this kind of study was repeated in 1998 (Table 3).

Evaluation of CAST Data for Residue Violation Rate

The total number of calf kidneys with violative concentrations of antibiotics and sulfonamides on the basis of CAST screening test results from 1984 to 2000 were derived from the yearly Domestic Residue Data Book of the FSIS National Residue Program. The violation rate in kidneys for each year was calculated per 100 calves slaughtered, and the yearly rate is presented graphically (Figure 3).

Results and Discussion

The varying zones of inhibition of \( B. \) \textit{megaterium} on CAST plates by various concentrations of SMZ at different temperatures indicate that the incubation temperature affects the size of the ZI (Table 1). The sensitivity of CAST to SMZ increases as the incubation temperature rises, allowing detection of the drug at a lower concentration. For example, a ZI by 1.0 ppm SMZ occurs only at 45°C. However, at 50°C, the growth of organisms on test plates becomes inconclusive and the ZIs lose their definition and cannot be measured accurately. Tests performed with cotton swabs containing aminoglycosides, beta lactams, macrolides, tetracyclines, and sulfonamides show that response by CAST to these antibiotics is greater than by STOP, i.e., CAST is more sensitive than STOP (Table 2). This became evident when SMZ at a 0.1 ppm concentration produced inhibition on the CAST plate, whereas a concentration of 4.0 ppm of any of the sulfonamides did not produce inhibition on STOP plates.

Bioassays and chemical analyses performed at the laboratory on liver and muscle and on CAST screen test positive kidneys show that, as expected with a target tissue, the percentages of violations were higher with kidney samples than with liver and muscle (Table 3). Compared to 1990 data, the percentage of positive kidney tissues for antibiotic or sulfa drugs decreased from 24.1 to 19.2 during the following years. The rate in liver during those years also decreased very slightly from 10.6 to 9.6, whereas the rate in muscles increased from 2.3 to 4.6. The rise in rate for muscles may have resulted because fewer samples were analyzed in 1998 than in preceding years. The approximate ratio of kidney 444
versus muscle positives in 1990 was 10:1. By the next year, the ratio decreased to 4:1 and remained roughly the same even after 8 years, until 1998 (23). The ratio of kidney versus muscle positives indicates that, during these years, the rate of carcasses with antimicrobial positive residue, tested with CAST, remained the same. Based on that fact, it can be deduced that the performance of CAST remained unchanged and has helped the Residue Control Program of FSIS to remove animal carcasses injurious to human health at the same level.

The incidence rate of kidneys with violative levels of antimicrobial residues as detected by CAST indicates that the rate of 4.9% in 1984 gradually declined to 0.5% by 2000 (Figure 3). This indicates that the use of CAST by FSIS has caused awareness in packers and producers about the agency program, and farmers to become cognizant of the proper use of drugs and medicated feed, including adherence to recommended withdrawal times for medicated feed under better animal husbandry practices.

As early as 1981, federal inspectors began to find bob veal calf carcasses with stomach discoloration during postmortem examination. The condition was commonly referred to as “green gut” (5). The color was suspected to be the result of misuse of sulfonamide medications, and the problem appeared to be widespread throughout the Northwest, Mid-Atlantic, and upper Midwest.

FDA tolerance levels for SZM, sulfadimethoxine, sulfachloropyridazine, sulfabromomethazine, and sulfathetoxypyridazine in edible tissues of cattle is 0.1 ppm. This chloropyridazine, sulfabromomethazine, and sulfanamide medications, and the problem appeared to be widespread throughout the Northwest, Mid-Atlantic, and upper Midwest.

FDA tolerance levels for SZM, sulfadimethoxine, sulfachloropyridazine, sulfabromomethazine, and sulfathetoxypyridazine in edible tissues of cattle is 0.1 ppm. This tolerance level was used in FSIS regulations to ensure safety of meat and poultry. To support the FSIS Residue Control Program, CAST was developed and introduced in slaughter establishments to screen bob veal calves with green gut caused by misuse of sulfonamides. Though the lower LOD of CAST was higher than the tolerance level for sulfonamides, it was better than STOP, which was 4 times less sensitive and would have missed calves with sulfonamide residue levels injurious to human health. However, since 1998, disposition of suspected bob veal carcasses are made on the basis of chemical analysis performed in FSIS laboratories and not on CAST results (23).

CAST is a modification of a microbial inhibition assay used for the detection of sulfa drugs and antibiotics in milk (24). These modifications to the microbial inhibition assay include a change in the test material from milk to kidney fluid, application of tissue fluid by a sterile cotton swab and not by a paper disc, streaking of B. megatatarium spores directly onto a test plate instead of agar seeding of test organisms, and an increase in the incubation temperature from 37°C to 45°C. This increase in the ZI by SMZ at higher temperatures may have resulted from the increased sensitivity of the heat-stressed germinating cells and from the interference in cell biosynthetic characteristics during cell outgrowth by SMZ (25). These modifications resulted in a rapid, easy-to-perform screen test for use at slaughter establishments, specifically for detecting veal calf carcasses with violative levels of antimicrobial residues. According to the Federal Food, Drug, and Cosmetic Act [21 USC 360 (b)], violation level is defined as the “concentration that exceeds the FDA tolerance limit for an antimicrobial drug in a specific species and tissue.”

The working principle of CAST is based on the microbial inhibition assay (26). Because kidney tissue is the target tissue for many antimicrobials, their presence can be detected in the kidney fluid. If the concentration of the antimicrobial in the kidney fluid is above a certain level, then the fluid will inhibit the growth of a susceptible organism. Thus, a kidney swab causing microbial inhibition is suspected to contain a residual antibiotic, a sulfonamide, or both. The acceptance criterion of an 18 mm (horizontal plus vertical measurement of inhibition around a swab) or greater ZI by a swab was based on field data obtained from kidney tissues containing antimicrobial residues at violation levels. Additionally, to verify CAST results observed at the slaughter establishment, muscle, liver, and kidney tissues from the same carcasses were periodically verified by laboratory bioassay. The compiled laboratory data for 1990–1994 show that the CAST-positive kidneys ranged from 61 to 72%, the range of positives for liver was 20–28%, and the range of positives for muscle was between 7 and 13%. To determine whether there had been a shift in the rate of positive tissues, the 1998 CAST data were analyzed. The data indicated that the rate of detection in liver and muscle was higher ($p > 0.5$) in 1998 than in 1994. One reason for a higher rate in 1998 could be that analyses of meat tissues for antibiotics in 1994 were conducted in 3 different laboratories, whereas in 1998 the data were analyzed in only one laboratory. Thus, the variation in analysis was better controlled in 1998. Second, due to the increased sensitivity of the bioassay plates to meet regulatory requirements (20), the rate of detection of violative tissue increased. Additionally, the use of many new antimicrobials in animals started in the 1990s. However, analysis of the concentrations of antimicrobials in muscles showed that the residue concentrations were not in violation. The data for 1998 show that there were no muscle samples in violation. With 4 kidney samples found in violation for antibiotics and 1 liver sample in violation for sulfonamide, the overall violation rate for kidney was 0.8% and for liver was 0.3% (23).

The FSIS residue data for CAST show that, since 1996 (13), <1.0% of kidney samples from veal calves contained antimicrobial residues at a violative level, except in 1998 (23), when the rate was 1.5% (Figure 3). Therefore, encountering muscle tissue with antibiotics or sulfonamides at a violative level is rare except in muscle from an injection site.

Prior to the use of CAST in slaughter establishments, the test was thoroughly evaluated for accuracy, reproducibility, and sensitivity. Studies showed that a kidney tissue fluid sample producing an 18 mm or greater ZI on CAST plates suggested that the carcass was very likely contained a violative level of drugs (3, 4, 10).

The impact of CAST on the decline of residue violations in bob veal calf tissue is clearly evident in Figure 3. The incidence rate was 4.9% at the time CAST was first introduced in the field in 1984. During the 10 year period of CAST usage,
the rate decreased to 1.3% by 1994, with one spike of 2.67% in 1989. This rise could be attributed to the new use of drug boluses in healthy calves prior to slaughter and then not allowing enough withdrawal time for the antimicrobials to clear the tissues.

The trend for residue violation to decrease (Figure 3) clearly shows that the use of the CAST as a screen test has helped to reduce the indiscriminate use of antimicrobial chemicals in calves. Additionally, it permits final carcass disposition within 24 h and has saved FSIS resources for handling and sending samples for laboratory analysis.

Conclusions

The ability of CAST to detect a wide spectrum of antimicrobial agents in bob veal carcasses makes it a valuable tool for keeping chemical residues out of the human food chain. CAST is a simple screening test for detecting multiresidues in bob veal calf tissues, though the test itself does not specify the nature or the concentration of an antimicrobial in a tissue. To identify and to determine the concentration of a particular antimicrobial residue, laboratory analysis of the tissue is necessary (Table 3).

Analysis of Residue Program data for the last 16 years (1984–2000) indicates that 98% of all bob veal calves tested were found free of antimicrobial residues, but the remaining 1–2% were a potential human health risk. Use of CAST has been a valuable aid to the FSIS mission of protecting public health. Overall, the CAST testing program may be one of the most successful and efficient regulatory program initiatives for controlling antimicrobial residues in food animals.

Acknowledgments

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We dedicate this paper to the memory of the late Richard H. Reamer (USDA/FSIS, Beltsville, MD).

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(19) Federal Register (1996) 51, 4849