# RAPID TESTS FOR RESIDUE CONTROL PROGRAMS.

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## Introduction

In the decade of the 1980s, heightened awareness about food safety demands more analytical capability and capacity. About five years ago, FSIS commissioned the national Academy of Sciences (NAS) to assess how effectively the agency was accomplishing its mission. In 1985, the NAS issued its report. The report points out, an we concur, the most important public health responsibility of FSIS is the risk associated with bacteria and chemical residues, neither of which can be detected by eye or organoleptically. Included in that NAS report, were recommendations for higher levels of sampling to detect residues and to provide greater assurance of a safe food supply for consumers. To define our specific actions, FSIS prepared a response in 1986, titled "FSIS Future Agenda". Our actions for residue control are clearly stated in that report. The need to rethink the program design was evident. Emphasis had to be placed on developing and using rapid testing procedures.

Important factors for using rapid test methods include their ability to analyze a relatively large number of samples in a unit of time, and their robust nature. This latter characteristic encourages the use of these qualitative and semi-quantitative methods in non laboratory surroundings where tests may often be performed by individuals not experienced in analytical chemistry techniques. However, methods performed in non laboratory surroundings places constraints and needs on certain types of methodology. It a) limits use of certain types of equipment, instruments, and reagents, b) requires methods to be written in simple, unambiguous instructions that will enable a tester to correctly prepare the test material, conduct the analysis, interpret and report the test findings, and c) requires developing process controls defining critical steps in the test procedure.

It should be clearly understood that rapid screening methods generate useful but potentially imperfect information. The focus of these methods is to detect the presence or absence of a compound or class of compounds at some designated level of interest and are often based on non instrumental techniques of analyte determination. Thus, results for a given sample may not be as reliable as quantitative or confirmatory methods unless there is corroborating data. A caution then, would be that proposed regulatory action based on individual positive results is supported by quantitative or confirmatory methods as determined by the uncertainty of an analytical result.

To ensure analytical reliability for regulatory programs, performance characteristics must be determined by multilaboratory evaluation. Minimum standards should fit the needs of specific program requirements. The principal attributes we consider relevant for analytical methods are specificity, precision, systematic error, and sensitivity. With rapid test systems, we also want a degree of performance that routinely achieves parrallel curves for standard solutions of an analyte and extracts of analyte added to a sample. The sensitivity we seek in a method is its ability to discriminate small differences in analyte concentration. Accuracy requirements for screening methods, the characteristics of false negatives and positives will be a major factor in defining their operating range.

Other attributes for rapid tests are disirable. The method should be a) rugged or robust– relatively unaffected by small analytical deviations from optimal parameters for time, temperature, concentrations, amounts of reagents, b) cost effective – use of relatively common reagents and instruments as well as using resources efficiently, c) relatively uncomplicated – using simple, straightforward mechanical or operational procedures, d) portable – transferable form one location to another without loss of established performance characteristics, e) resource efficient – capable of handling a set of samples in a time effective manner, and f) safe – keeping in mind that testers may have limited analytical skills and may not have well equipped and ventilated working areas.

Integrating rapid test methods into residue control programs may depend on perceived violation rates and public health or food safety issues. One possible situation – when the incidence of violations is known or high, is exemplified by abattoir testing for sulfanamides in swine.

## MATERIALS AND METHODS

Sulfonamides have a long history of human and veterinary use. Sulfamthazine (SMZ), in particular, has been used extensively in swine production because of its efficacy, versatility, low cost, and long history of successful therapeutic and prophylactic treatment. Estimates suggest that up to 80 percent of all hogs marketed in the United States have received some form of sulfa medication.

Throughout the 1970's and 1980's violative SMZ residues in swine continued to exist at an unacceptable rate of from 4 to 13 percent as measured in the USDA National Residue Plan. Efforts throughout this time frame did not achieve and maintain a violation rate considered acceptable by todays public health standard.

We had developed a very practical quantitative method for sulfonamide drugs that has been successfully validated and is an official association of Official Analytical Method for sulfonamide drugs in animal tissue. This method is basically a five step procedure using thin layer chromatography with quantitation by scanning densitometry following colour development using flourscamine. An internal standard, sulfapyridine, is used for quantitation of many sulfonamides including sulfamethazine (Thomas, et al., J. Assoc. Off. Anal. Chem. 66, 884–892 (1983)). Though it is a practical quantitative method for laboratory use, it is not for use in an abattoir. Wishing to use this developed technology, we decided to evaluate its application to field use. In 1982 and 1983, we conducted three controlled swine feeding studies at the USDA Meat Animal Research Center (MARC) to explore the relationships, if any, between sulfamethazine concentrations in muscle, liver and kidney tissues, blood and urine. Based on the three studies, using approximately 120 market weigth boars and gilts, tissue – fluid relationships were developed (Randecker, et al., J. Food Prot. 50, 115–122 (1987)).

Results of these studies are summarized in Table 1.

Table 1.
Tissue – Fluid Relationships of SMZ Residues in Swine.

| \Tissue Fluid\ | Muscle | Liver | Kidney |
|---|---|---|---|
| Serum | 0.24 | 0.90 | 0.53 |
| Urine | 0.08 | 0.27 | 0.16 |

Using these data, options were considered for using serum and urine for a field test coupled with the proven thin layer chromatography. Urine proved to be the fluid of choice because it was a) easier to collect and identify in an abattior, b) resulted in a simpler and shorter analytical method, and c) provided useful multipliers for predicting tissue residues (approximately 4:1 ratio between urine and liver concentrations and 12.5:1 for urine and muscle tissue). The disadvantage was that SMZ concentrations in urine had a larger coefficient of variation (CV) than serum within individual feeding groups in the three studies. Even though our development effort focused on urine, practical methods for both fluids as well as medicated feed were realized.

The result of this major undertaking resulted in the development and eventual implementation of the Sulfa On Site (SOS) test. The SOS test may be performed by inspectors providing same day results. The test uses a 10 channel thin layer silica gel chromotography plate having a preadsorbent area. Twenty microliters of urine collected from the bladder is adsorbed onto the preadsorbant layer. On separate channels are placed two concentrations of SMZ standard (0,4 and 1,3 mg/kg). The samples are dried with a small hair drier, placed in a chromatography jar and eluted with methanol to the upper boundary of the preadsorbant layer, and dried again. Next the plate is developed with a second solvent, ethyl acetate, to a distance of 2 cm. above the boundary of the preadsorbant layer, dried again and sprayed with a solution of fluorescamine in acetone. After 15 minutes in the dark, the TLC plate is observed under ultra violet light to interpret results. A set of six samples and the two standards can be run in about 30 to 40 minutes. When the sample bands have a similar or greater intensity than the standards, SMZ residues are present at a level of interest.

The test is calibrated so that individual sample readings predict whether the corresponding liver or muscle tissue from an animal are violative. Because the SOS test uses a low and high concentration standard, results can be reported in four categories, each category predicting varying concentrations of SMZ residues. A result of "0" indicates no detectable residue, a "1" indicates SMZ is present in tissue but at a concentration of less that 0.1 mg/kg in liver and muscle, a "2" predicts concentrations greater than 0.1 mg/kg in liver and less than 0.1 mg/kg in muscle, and a "3" predicts a concentration of greater than 0.1 mg/kg in liver and muscle.

Based on observations of visual acuity, most individuals can clearly detect a urine sample containing 0.2 mg/kg SMZ.

Although the SOS test has been designed to detect SMZ, it will also detect the presence of several other sulfonamides having different Rf values. Estimates of tissue concentrations with these other sulfonamides has not been attempted because the necessary tissue–fluid relationship studies have not been conducted.

Equipment needs for the test kit were met using commercially available supplies. The major non consumable items included a hair drier, portable ultra violet light and light box, chromatography jars, and test tube holder. Consumable items include 10 channel silica gel chromatography plates, 15 ml plastic centrifuge tubes, disposable micropipets, SMZ standards, flourescamine solution, and solvents. The kit was packaged originally in sets for 600 tests. Smaller sets of replacement kits of consumable items are now available.

## RESULTS

Data from the seven in plant studies in five states (over 1100 field test results) provided an opportunity to optimize the test method and to confirm the results from the three MARC studies (USDA unpublished report). In addition to our studies, a field trial was conducted with the cooperation of the Iowa State Veterinary School. Students were taught to perform the SOS test on hog urine and finishing feeds. Producers were informed of the availability of the testing facility and requested to bring samples of urine and finishing feed from their herds. 178 producers responded with guaranteed anonymity of samples. A total of 337 urine samples and 177 feed samples were analyzed. Excellent correlation was noted between urine tests and finishing feeds. In our Dubuque, Iowa, abattoir study, veterinarians with no prior experience conducted the SOS test for SMZ in hog urine, using the self instruction guidebook prepared by our Program Training Division. More than 90 percent of the time, inspector results agreed exactly with the results of an experienced analyst.

In all these field trials, results were compared with quality assurance analysis performed in our laboratories with the quantitative sulfonamide method. For the urine samples tested as negative for SMZ or detectable but below 0.4 mg/kg in urine, the prediction accuracy was greater than 99.6 percent in liver and 99.8 percent in muscle. Thus, there were less than 0.5 percent false negatives. Liver and muscle violations are predicted with less accuracy due to difficulty in visual discrimination around the 0.4 mg/kg low standard where most individuals cannot readily discriminate differences in fluorescence brightness when sulfonamide concentrations differ less than two–fold. That is, some may interpret a 0.4 mg/kg standard as less than the low standard while approximately an equal number will estimate the result as greater than the low standard. The accuracy for carcass violation (results indicating SMZ concentrations greater than the high satndard) was greater than 80 percent. These results warranted a validation study using plant inspectors.

A 30 plant validation study was undertaken to evaluate test performance with trained field inspectors. We traveled to several swine abattoirs throughout the United States and personally trained designated inspectors and plant personnel. The study was planned to cover 100 working days. During this period, each inspector was to test six animals per day. Urine samples that indicated SMZ or another sulfonamide and a percentage of negative samples were shipped to a laboratory for quality assurance testing. Approximately half way through the study, we suspended the validation study because inspectors trained in the SOS test in some of the plants had been reassigned. The study was completed with inspectors in 18 of these avattoirs after inspectors demonstrated proficiency on a set of 18 check samples.

The same kind of data analysis used to predict SOS test accuracy in the previous field trials was applied to the data from the inspector in plant feasibility study. Based on over 2600 data points, for samples reported as non detectable or less than the concentration of the low standard, inspectors had a 94.5 percent accuracy on residues in liver and 99.3 percent accuracy on predicting non violative SMZ residues in muscle. For residue violations in liver and muscle tissue compared to the high standard (1.3 mg/kg in urine), accuracy was 81.5 percent in liver and 70 percent in muscle.

Based on this and supporting data we recommended approval of the SOS test for in plant screening for clearing animals with non detectable or detectable SMZ residues but below a level of interest. As of April, 1988, this has been an official FSIS test. The SOS test is being used to pass hogs tested as non detectable for SMZ or less than the concentration of the low standard. Animals testing as suspect residue violations are retained and tissue samples sent to a laboratory for quantitative analysis. Those samples with tissue concentrations greater than the tolerance (0.1 mg/kg in tissue) are condemned.

In February 1988, FSIS announced plans to intensify regulatory efforts following the publication of the National Center for Toxicological Research linking SMZ to thyroid gland tumors in laboratory animals and subsequent actions by Japan retaining United states pork exported to Japan. The plan included a system for mandatory animal identification, stepped-up testing of hog carcasses in abattoirs using the SOS test, a regulation for lot testing, voluntary testing of live hogs, and development of improved analytical methods.

Part I of the intensified residues testing consisted of collecting muscle samples from two hogs from each of the 100 largest swine abattoirs (representing about 98 percent of hogs slaughtered). The hog carcasses sampled were retained for disposition until laboratory results were available. Part I testing continued in effect at each of the 100 abattoirs until veterinarians in individual plants were trained in the SOS test. During Part I, 1618 muscle samples were tested and 29 contained violative residues of SMZ in muscle tissue. Note, however, that residue violation data from the National Residue Plan is based on random sampling on a yearl basis and on residue violations in liver tissu

rather than muscle tissue. Thus, results fro these two programs provide differe information.

Part II of the program consisted implementing the SOS test in each of th 100 largst plants within a two mont timeframe as inspectors were trained an supplies distributed. The phase in began April 4, 1988 and was completed by May 2 1988, when sufficient numbers of traine inspectors (216) permitted the SOS testin program to become operational. Each workin day in the participating plants, six urine were collected and tested for sulfanomid residues. Carcasses tested were retained un inspectors completed the test. If the urin test indicated violative residues in liver muscle, the offal was condemned, carcasse were retained, and muscle samples sent fo laboratory analysis. Disposition was based the laboratory analysis.

Results of the testing program have bee gratifying. In 1988, we performed 86,510 SO tests and through June 1989, we hav completed 141,056 tests. The number muscle violations, by laboratory analysis, 95 543. Based on the SOS test results, 95 percent of the animals tested in 1988 wer reported to contain no detectable sulfonamid residues, while an additional 3.2 percen contained sulfonamides but less than the 0. mg/kg tolerance in liver and muscle.

Equally encouraging, the month by mont trend in 1988 was in a downward directio In June 1988, the first full month of part of the program, 66 violations were noted 10,233 samples tested. In December 1988, 2 violations were reported from 10,860 samples SMZ residue violations from the 1988 Nation Residue Program declined to 1.42 percent the lowest ever. For comparison, the SM residue violation rate for the previous ye was 3.8 percent. This overall reduction sulfonamide redidues is due to several factor but clearly, the availability of the in pla test contributed signigicantly to the resu of this nationwide effort.

CONCLUSIONS

Part of the enhanced sulfonamide progra developed in 1988, called for improve analytical methods. Presently, were actively pursuing two different technologie

to supplement the current SOS test–computer assisted chemical analysis and planar chromatography.

Together with Dr. James Callis at the University of Washington Center for Process Analytical Chemistry, we are exploring approaches for developing integrated analytical approaches to in plant testing. They have developed non–invasive spectroscopic techniques that monitor on–line production conditions. We initially saw opportunities of this technology to facilitate interpretation of SOS test results, relieving inspectors from subjective interpretation based on visual acuity.

By combining a small television (vidicon) camera, a non–scanning fluorescence spectrometer, and an image capture board with a personal computer, SOS plates can be read more precisely, and automatically, with minimal operator intervention, to produce self–documentation of the test results. Because the system is coupled with a lap–top portable computer, the entire instrument is small enough and rugged enough to fit under an airline seat, enabling it to be carried from site to site, as needed.

We intend to further explore this approach through field studies and other applications to rapid field tests for supporting slaughter inspection. By modifying the detector, or adding visible and intra red light sources, coupled with the capabilities of available portable computers, this field portable system could be applied to a wide range of on–site tests. Examples include aflatoxin screening, multi–variant analysis of simple chromatography for pesticides, other veterinary drug classes and environmental contaminants, analysis of gel immunodiffusion plates, microbiological agar plate counting, and ELISA microtiter tests.

Testing sensitivity, accuracy, reproducibility of results, and improved data quality control and management in residue and microbiological testing can be enhanced by using this potentially multi–purpose instrument. For example, test results could be transmitted electronically to a central data collection facility or to existing residue data bases with a minimal number of key strokes, greatly reducing the need for inspectors to fill out forms by hand. In addition, mailing of forms and hand entry of data at a processing center would be reduced. Clearly there is much development work to be done and we are ready to proceed.

Planar chromatography is simply a horizontal application of classical thin layer chromatography. It does, however, have some distinct applications to the SOS test and other non–laboratory tests involving chromatographic applications. The present chromatography system in the SOS test kit requires the use og approximately 15 to 20 ml of solvent and glass chromatography jars. In many abattoirs there are minimal facilities for running rapid tests. Inspector facilities may lack adequate ventilation systems to handle solvent vapors or disposal of waste solvent, creating safety concerns. We carefully reviewed this with the SOS test from the start and had environmental experts evaluate the test system. They were satisfied with the safety of the test system. Nevertheless, reducing solvent use as well as space needs and ruggedness of test components were obvious advantages to us. The system we are evaluating was designed by the Cera Company. The chromatographic unit is made of teflon with a heavy glass cover plate. The machined teflon base is designed with small sample wells that allow chromatography to be conducted using no more than 3 ml of solvent. Further, with two wells, the same unit facilitates chromatography with the two solvents needed for the SOS test, thus eliminating the need for two chromatography jars. There is also built in versatility that allows the system to accomodate rigid glass as well as flexible based chromatography plates. We think there is great potential for this approach for both laboratory as well as field applications for the SOS test and others. We are exploring both applications.

Clearly, new technologies are presenting new challenges and opportunities for rapid test development for regulatory programs. Our task is to optimize and apply their benefits to improve our effectiveness in meat and poultry inspection, providing a safe and wholesome meat supply at minimal cost to consumers.