CHAPTER 35. DETECTION OF ANTIBIOTIC AND SULFONAMIDE RESIDUES IN MEAT TISSUE BY COMMERCIALLY AVAILABLE IMMUNOASSAY KITS

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35.1 Introduction

In recent years, the application of immunochemical methods for detecting veterinary drug residues in animal tissue has increased. These methods are based on highly specific antigen-antibody reactions on a solid phase matrix involving conjugation of an enzyme to the drug analyte and specific antibody directed against the analyte. Advantages of enzyme immunoassays include sensitivity (usually in the ng/ml range), simplicity of test performance, stability of reagents, lack of radioisotope use and associated hazards, potential for automation, and relatively inexpensive equipment.

In direct competitive enzyme immunoassays, enzyme labeled drug antigen and unlabeled drug antigen (sample analyte) compete for limited antibody binding sites. Specific antibody is generally bound to a solid phase support. The amount of enzyme labeled drug that binds to antibody is inversely proportional to the amount of unlabeled drug antigen (analyte) present in the tissue sample, which also competitively binds to the same antibody. Upon addition of substrate to the reaction mixture, to provide a visible indication of the test reaction, the amount of colored end product produced is inversely proportional to the amount of unlabeled drug analyte bound to the antibody. Thus, positive reactions indicating the drug's presence in the sample are generally indicated by no color change, while negative reactions indicating absence of the drug analyte are usually colored products. The exact color of the end product depends upon the specific substrate - chromogen system used in the particular assay. The applications of direct competitive enzyme-linked immunosorbent assays (ELISA) have provided additional support to the FSIS regulatory programs by enabling the detection of drug residues in food animal tissues at appropriate levels.

Presently, there are a number of screen test kits commercially available for detecting the presence of antibiotic and sulfonamide residues. However, regulatory action cannot be based on screen test results alone, since they are not quantitative, do not relate to biological activity of the detected drug and they are not considered to be absolutely definitive and confirmatory in nature. The presence of antibiotic residues, therefore, must be confirmed by bioassay and/or chemical methods, when a chemical method exists.

35.2 Commercially Available Test Kits

Currently there are commercially available, immunoassay, screen test kits, in several different formats, for such antimicrobial agents as:

penicillin (β-lactams)
ceftiofur (cephalosporin)
chloramphenicol
gentamicin
sulfonamides
tetracyclines
neomycin

Many of these test kits, however, were originally developed for specific application in bulk milk tank testing for antimicrobial residues. Before any of these kits can be used in an FSIS Laboratory for testing meat tissue extracts for antimicrobial residues, they must first be thoroughly evaluated to determine their suitability and applicability with regard to appropriate performance characteristics. They must perform in a manner to meet minimum sensitivity detection levels for the drug in question relative to that drug's established tolerance level, be specific, show excellent lot-to-lot reproducibility, have stability over the reported shelf life of the kit, and also have very low (0-5%) false positive and false negative reaction rates. Non-government users of this Guidebook must assume individual responsibility for evaluating commercial test kits for their applicable suitability with regards to the above performance parameters.

35.3 Equipment

This generic list applies to all test kits. Depending on the exact kit used, other supplies might be required.

a. Tekmar stomacher®, Model 400 (Tekmar Company, Cincinnati, OH)
b. Eppendorf centrifuge, Model 5412 (Thomas Scientific Co., Swedesboro, NJ)
c. Timer
d. Tekmar strainer bags, 18 oz capacity. (Tekmar Company, Cincinnati, OH)
e. Micro centrifuge tubes, 1.5 ml volume. (Thomas Scientific Co., Swedesboro, NJ)

35.4 Reagents

a. 0.1 M phosphate buffer, pH 8.0 (± 0.1). Dissolve 16.73 g dibasic potassium phosphate and 0.523 g monobasic potassium phosphate in distilled water and

dilute to 1 liter with distilled water. Check pH of the nonsterile buffer before autoclaving. If necessary, adjust by the dropwise addition of 0.1 N HCl or NaOH. Autoclave for 15 minutes at 121°C and 15 lbs pressure.

b. 0.1 M phosphate buffer, pH 4.5 (± 0.1). Dissolve 13.6 g monobasic potassium phosphate in distilled water and dilute to l liter with distilled water. For pH adjustment, proceed as in (a) above.

c. 0.1 M phosphate buffer, pH 6.0 (± 0.1). Dissolve 11.2 g monobasic potassium phosphate and 2.8 g dibasic potassium phosphate in distilled water and dilute to l liter with distilled water. For pH adjustment, proceed as in (a) above.

d. U. S. Pharmacopeia (USP) antibiotic and sulfonamide standard reference materials

35.5 Tissue Extraction Procedure

This procedure generally applies to all test kits:

a. Weigh out 10 g of sample (muscle, kidney, or liver tissue) into a sterile container.

b. Place the sample into a labeled Tekmar strainer bag.

c. Add 40 ml of appropriate phosphate buffer for the antibiotic or sulfonamide residue under evaluation.

d. Place strainer bag in a Tekmar stomacher® and stomach for 30 seconds for kidney or liver and 60 seconds for muscle tissue.

e. Allow the extract to settle for 45 minutes.

f. Place 1.5 ml of the settled extract into a labeled micro centrifuge tube.

g. Centrifuge for 10 minutes in an Eppendorf micro centrifuge at maximum speed.

h. Pipette supernatant fluid into another labeled test tube avoiding any fat and debris.

i. The 1:5 extracts prepared for bioassay analysis (Chapter 34) can be used instead of performing steps a through e.

35.6 Performing the Assay

Perform the assay procedure according to the specific test kit manufacturer's directions if no modifications were found to be necessary, or according to any specific instructions provided by the Microbiology Division, OPHS, for a particular test kit, when extensive protocol modifications were necessary.

35.7 Reporting and Confirmation of Screen Test Results

All positive screen test results should initially be reported as a presumptive positive finding. All samples presenting a positive screen test result must be subjected to confirmatory testing by the bioassay procedure or an appropriate chemical analysis procedure, if available for that particular drug, to confirm the drug's identity and determine it's quantitation. All sulfonamides must be confirmed by appropriate chemical methods. Final violative result reports must be based on confirmed drug quantitative levels present above that of established tolerance levels for that drug in a specific animal slaughter class.

35.8 Quality Assurance Procedures

a. Maintain a written log of all kits purchased, used and appropriate dates.

b. Test kits must be stored under refrigeration (4-8°C). Do not freeze.

c. Upon receipt of new test kits, perform positive and negative control testing at appropriate drug concentration levels.

d. Do not mix reagents and test components from kits with different serial numbers or from different manufacturers kits that detect the same analyte.

e. Do not use kits past their expiration date.

f. Use a separate pipet and test device for each sample.

g. Before performing the test, allow all reagents to reach room temperature. If the room temperature is not within the range of 18-29°C (65-85°F), perform test in another area within the proper temperature range.

h. Observe all test time intervals accurately by using a timer.

i. Two weeks before test kit expiration, perform positive

and negative control tests at appropriate drug levels to assure proper kit performance as expiration approaches.

j. U.S. Pharmacopeia (USP) standards of antibiotics and sulfonamides at appropriate quantitative levels should be used.

k. Record the results of all positive and negative control tests in a log book.

35.9 Selected References

Agarwal, V. K. 1992. Analysis of Antibiotic/Drug Residues in Food Products of Animal Origin. Plenum Press, New York, NY 10013.

Boison, J. O., and J. D. MacNeil. 1995. New test kit technology, p. 77-119. In H. Oka, H. Nakazawa, K. Harada and J. D. MacNeil (ed.), Chemical Analysis for Antibiotics Used in Agriculture. AOAC International, Gaithersburg, MD 20877.