Committee on Drugs and Related Topics

Drug Residues in Foods, Diagnostics and Test Kits

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Summary

This year, 8 commercially available rapid test kits have been certified by the AOAC Research Institute (AOAC–RI). These include: 2 beta-lactam test kits, Charm SL Beta-Lactam Test Kit (Charm Sciences, Malden, MA) and Parallux Beta-Lactam Test Kit (IDEXX, Westbrook, ME); and 2 rapid tests for toxins, RIDASCREEN® FAST DON (r-Biopharm GmbH, Darmstadt, Germany) for the detection of deoxynivalenol (DON) in cereals and RIDASCREEN FAST T-2 Toxin Assay (r-Biopharm GmbH) for the rapid detection of T-2 toxins in corn, wheat, and mixed feeds. Verification of the T-2 Toxin assay’s performance claims to quantify T-2 toxin in corn at or above 150 ppb and in mixed feed at or above 300 ppb was confirmed through independent testing by the Canadian Food Inspection Agency (1). Four test kits for food-borne pathogens were also certified: Detex System MC-18 (Molecular Circuitry, King of Prussia, PA) for E. coli O157 including H7; EIA Foss Listeria ELISA (Foss Electric, Hillerød, Denmark) for the detection of Listeria spp. (with the exception of Listeria grayii); BAX (Qualicon, Wilmington, DE) for screening Listeria monocytogenes in a variety of foods, and BAX (Qualicon) for screening E. coli O157:H7 in ground beef.

Last year, the AOAC–RI published a list of 35 rapid test kits that had been certified for screening various analytes in foods under the AOAC® Performance Tested MethodsSM Program (PTM; 2). These test kits designed for use by regulators, producers and processing establishments are used for the detection of bacterial, biochemical, antibiotic, hormone, toxin, and chemical contamination in foods to reduce or prevent the introduction of contaminated foods into the food chain. One of these test kits, the Charm SL Beta-Lactam test kit for the detection of amoxicillin, ampicillin, cephapirin, cefitiofur, and penicillin G in bulk tank bovine milk, has undergone extensive validation in the PTM program (PTM 990801) and a manuscript describing this study is ready for publication in the J. AOAC Int. Another manuscript describing the validation study conducted for the Parallux™ beta-lactam test kit is also ready for publication. This General Referee is working very closely with the AOAC–RI to expedite the publication of these studies. This will fulfill one of the objectives recently discussed by the AOAC–RI Board of Directors to develop a new format for validation study reports in the PTM program. The publication of these studies will also enhance the initiation of collaborative studies involving rapid test kits.

Five commercially available rapid test kits, most of which had been previously certified by the AOAC–RI, were evaluated by Kroll et al. (3) for their suitability in detecting beta-lactam drug residues in milk at the Maximum Residue Limit (MRL). These test kits included the beta-STAR, Charm MRL, Delvo-X-Press® beta-Lactam II, Penzyme®, and the SNAP® test. Parallel tests were also conducted using 3 microbiological inhibition tests; Brilliant Black Reduction (BRT) MRL-Screening Test, BRT-Inhibitor Test, and Delvotest® SP. The study concluded that the beta-STAR, CHARM MRL, and the SNAP test were all suitable for detecting 12 beta-lactams in milk with European Union defined MRLs. The Delvo-X-Press and the Penzyme Tests were found to be less suitable as they were unable to detect some of the beta-lactams at their MRLs. Another AOAC–RI certified test kit, the Bioline Salmonella ELISA Test (PTM 960901) was evaluated by Bolton et al. (4) against the standard method described in the U.S. Food and Drug Administration’s Bacteriological Analytical Manual (BAM), for its use in detecting 20 different strains of Salmonella from a broad variety of serogroups in foods and feed. Bolton et al. concluded that this test kit is able to provide test results that are as reliable as those obtained using BAM and is also highly suitable for large test sample throughput screening. The method permits more than 500 test samples to be processed under manual conditions or over 2000 test samples to be processed with automation in a single day. Unlike the traditional methods that take 3–5 days to obtain test results, the Bioline ELISA test results may be available after only 36 h.

There are very few commercially available test kits that are designed for direct screening of animal tissues for veterinary drug residues in slaughterhouses. As such, scientists usually have to adapt a test kit that has been developed for milk testing for tissue testing. A classic example was the case where Belgium scientists (5) adapted the beta-STAR, a test kit that was developed for screening for beta-lactam drug residues in milk, to screen for ampicillin, amoxicillin, ampicillin, oxacillin, and cloxacillin residues in swine kidneys at the European Union MRL of 50, 50, 50, 300, and 300 ng/g. Henk Offeriens et al. (6) also reported using the PremiTest, a broad-spectrum standard diffusion test, to screen for tetracyclines, macrolides, aminoglycosides, sulphonamides, quinolones, penicillins and...
cephalosporins, and fenicos in fish fillets (eel, salmon, and trout).

McConnell et al. (7) from Randox Laboratories reported an interesting study in which they compared test results obtained on 2 proficiency test samples from the UK Food Analysis Performance Assessment Scheme (FAPAS) that had been analyzed by HPLC, HPTLC, TLC, LC/MS, and ELISA with those obtained with the Biochip. The biochip consists of a 1 cm² substrate on which discrete test regions have been constructed, each test region representing a different analyte. Biochip assays are immunosassay-based with chemiluminescent detection. Digital imaging technology is used to measure light signals generated from individual test regions. Nine biochips are imaged simultaneously, and the light signal generated is converted to provide the concentration of each chip parameter. It takes about 15–30 min to obtain the first test result while the other techniques take much longer to generate test results. Biochips developed so far include panels for detecting 12 sulphonamides, 8 anabolic steroids/beta agonists, and 9 drugs of abuse (9 panels). It is hoped that the company would extend the scope of analytes to include tetracyclines, aminoglycosides, and beta-lactams to make the test useful for slaughterhouse applications.

Recommendations

1. Beta-Lactam Residues in Milk. Delvotest: Vacant. Seeking Associate Referee. Interested scientists or collaborators are asked to contact this General Referee or AOAC INTERNATIONAL for information.

2. Beta-Lactams in Tissues by Quantitative Competitive Microbial Receptor Assay: Vacant. Seeking Associate Referee. Interested Scientists or collaborators are asked to contact this General Referee or AOAC INTERNATIONAL for information.

3. General Referee will work closely with the AOAC–RI and its expert reviewers to review validation protocol requirements and data for performance tested methods.

4. General Referee will work closely with the AOAC–RI and the J. AOAC Int. Editorial Board to expedite the publication of PTMs.

References

(1) Anon (2000) J. AOAC Int. 83, 8A
(2) Anon (1999) J. AOAC Int. 82, 80A-92A

Drug Residues in Animal Tissues

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Summary

The topic continues to be well represented in research publications in J. AOAC Int., with 21 papers published since the preparation of last year’s report. As in recent years, the majority of publications dealt with methodology for antibiotics, primarily using liquid chromatography (LC). These included multiresidue LC methods for penicillins (1) and macrolide (2) antibiotics in muscle tissues. The penicillin method detects 8 penicillins in beef, pork, and sheep muscle, with limits of determination from 25–75 ppb (ng/g, 1), while the macrolide method detects tilmicosin, tylosin, spiramycin and neospiramycin in beef, pork and poultry muscle with limits of detection from 15–30 ppb (ng/g; 2). The results of a collaborative trial of a method for ampicillin residues in pork muscle were presented by the developers of the penicillin multiresidue method at the recent Euroresidue Conference (3) and submission of this study for review as an Official Method SM would be welcomed. Proceedings of Euroresidue IV are an excellent source of information on current research on veterinary drug residues and related issues.

A 5-laboratory validation trial of a method for tetracyclines in pork muscle and kidney using a polymer-based solid-phase extraction (SPE) cartridge for test sample cleanup was reported by the Associate Referee (4). This type of cartridge was found to give consistently higher recoveries of the tetracyclines, ranging from 76–87% from 0.2–0.6 mg/kg (ppm), than have previously been found when silica-based SPE cartridges have been used.

Continuing the interest in multiresidue methods, there was also a gas chromatographic (GC) method using electron capture detection for the determination of the related antibiotics chloramphenicol, florfenicol (and its metabolite florfenicol amine), and thiamphenicol in shrimp (5). Analytical recoveries were >80% for all the compounds at concentrations from 5–80 ppb (ng/g). A multiresidue LC procedure for the anti-parasite compounds abamectin, doramectin, ivermectin, and moxymectin in milk was tested in 2 laboratories, with recoveries >80% for 4 compounds at concentrations from

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1–30 ppb (ng/g; 6). LC determinative and LC with mass spectrometry (LC/MS) confirmatory methods for the same group of “meclins,” plus chloramphenicol, in tissue test samples were also reported (7, 8). Recoveries >70% were obtained for all the compounds at concentrations from 25–100 ppb (ng/g) in beef liver, following extraction and fluorescence derivatization (365 nm excitation, 465 nm emission), with LC analysis under isocratic conditions (7). All the compounds were confirmed at 25 ppb (ng/g) by LC/MS, using an APCI interface (8).

A comprehensive review of chromatographic methods for aminoglycoside antibiotics should be of interest to many regulatory analysts and offers good information on the selection of methods for this group of compounds (9).

Single residue LC methods were presented for amoxicillin in trout muscle (10), amoxicillin in beef, pork, chicken and tilapia muscle (11), fenbendazole in pork liver (12), nosiheptide (a polythiazole antibiotic) in meat and eggs (13), oxytetracycline in kidney and milk replacer (14), tilmicosin in milk (15), tylosin in milk (16), and p-toluenesulfonamide in fish (17).

Amoxicillin was extracted from trout muscle in aqueous solution by precipitation of organic materials with sulfuric acid and sodium tungstate, cleaned up on phenyl end-capped and polymeric SPE cartridges, derivatized with benzoic anhydride and 1,2,4-triazole, and detected by UV detection at 323 nm after LC separation on a C18 column using gradient conditions, with a detection limit of approximately 3 ppb (ng/g; 10). In a separate report, amoxicillin was extracted from beef, pork, chicken, and tilapia muscle tissues using buffer, cleaned up on C18 SPE cartridges, and analyzed following conversion to a fluorescent derivative (358 nm excitation, 465 nm after LC separation on a C18 column using gradient conditions, with recoveries from 82–94% (15). The procedure includes an extraction interface (8).

Beta-Lactam Antibiotics in Milk, LC Method

(1) Beta-Lactam Antibiotics in Milk, Delvetest: Associate Referee William A. Moats, U.S. Department of Agriculture–Agricultural Research Service, Bldg 201 BARC E, Beltsville, MD 20705, Tel: +1-301-504-7003, Fax: +1-301-504-8438, E-mail: wmoats@ggpl.arsusda.gov, has retired and did not submit a report. Continue Study. Seeking new Associate Referee. Any scientist or organization interested in participating in this topic is asked to contact this General Referee or AOAC INTERNATIONAL.

(2) Beta-Lactam Antibiotics in Milk, Delvetest: Transfer topic to General Referee for Test Kits (J. Boison). Continue Study. Seeking Associate Referee. Any scientist or organiza-
tion interested in participating in this topic is asked to contact this General Referee or AOAC INTERNATIONAL.

(3) Beta-Lactams in Tissues by Quantitative Competitive Microbial Receptor Assay: Associate Referee J. Scheemaker. Associate Referee resigned. Transfer topic to General Referee for Test Kits (J. Boison). Continue Study. Seeking Associate Referee. Any scientist or organization interested in participating in this topic is asked to contact this General Referee or AOAC INTERNATIONAL.

(4) Determination of Ivermectin in Animal Tissues: Vacant. Continue Study. Seeking new Associate Referee. Any scientist or organization interested in participating in this topic is asked to contact this General Referee or AOAC INTERNATIONAL.


(6) Improved Analysis of Tetracycline Residues in Swine Tissues Using Polymer-Based Extraction Cartridges: Associate Referee J. Zmudzki, National Veterinary Research Institute, Al Partyzantow 57, Pulawy 24100, Poland, Tel: +48-81-866-3051, Fax: +48-81-866-2595, E-mail: zmudzki@piwet.pulawy.pl, reported results of a 5-laboratory trial in the Journal. Continue Study.

(7) Determination of Clopidol in Chicken Tissues: Associate Referee Guo-Fang Pang, Qinhuangdao Entry-Exit Inspection and Quarantine Bureau of P.R. China, No. 39 Haibin Rd, Qinhuangdao 06600-2, People’s Republic of China, was appointed. His papers on analytical method and distribution of clopidol in chicken were submitted to the Journal for publication. Continue Study.


References

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(20) Moats, W.A. (1999) J. AOAC Int. 82, 1071–1076
(22) Pensabene, J.W., Fiddler, W., & Donoghue, D.J. (1999) J. AOAC Int. 82, 1334–1339