

**ESTABLISHMENT OF MODIFIED SCREENING TESTS TO DETECT  
ANTIMICROBIAL RESIDUES IN POULTRY MEAT, SHRIMP AND FEED**

**By**

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**A thesis submitted in conformity with the requirements for the degree of  
MASTER OF PHILOSOPHY**

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**2004**



**577154**

## ABSTRACT

Antimicrobials are used in food producing animals for treatment, prevention of diseases and growth promotion. There is a remote possibility of residues occurring in these antimicrobials in livestock and aquatic products, which is a current public health concern. Since the residues could occur due to unintentional use of antimicrobials in food producing animals, it can be prevented by Good Agricultural Practices (GAP), Good Veterinary Practices (GVP) and by monitoring the quality of food commodities. The monitoring of food commodities for residues will ensure the consumer safety in a nation and promote trade by fulfilling the requirements of the importing country.

Sri Lanka had been exporting broiler meat and shrimp to several countries not possessing a residue monitoring programme to ensure the chemical quality of the products. Therefore the objective of this study was to establish screening tests to detect antimicrobial residues in broiler meat, shrimp and animal feed.

Initially a modified version of the European Union Six Plate Test (EU-SPT) was established, which enables screening of six groups of antimicrobials, namely, penicillins, sulphonamides, streptomycin, erythromycin, tetracyclines and fluroquinolones.

The modified EU-SPT is a bioassay technique, which is carried out using *Bacillus subtilis* BGA (Merck), *Bacillus cereus* (ATCC 11778), *Micrococcus luteus* (ATCC 9341) and *Escherichia coli* (ATCC 11303) as indicator organisms. The EU-SPT was modified to use Mueller Hinton Agar (MHA) as the test medium at optimum pH for all indicator organisms except *B.cereus*, for which the Media No.8 was used. In order to improve the sensitivity for the detection of sulphonamides, trimethoprim (10µg/ml solution) was added to pH 7.2 medium seeded with *B.subtilis*. The antibiotic discs including penicillin G, sulphadimidine, streptomycin, erythromycin, chlortetracycline and ciprofloxacin were used as standards on respective assay plate. The SPT was validated using serial dilutions of antibiotics and accordingly Minimum Detectable Concentrations (MDC) were determined. All the MDC values obtained on SPT for the validated antimicrobials were below the recommended (Maximum Residue Limit) MRL of each antimicrobial.

Samples of broilers obtained from major processing plants, retail outlets and shrimp samples from export consignments of local shrimp processors. These were screened for residues using this established SPT. Of the 228 broilers sampled from processors only 15

birds (7%) contained antimicrobial residues. Out of the 64 random broiler meat samples purchased from retail outlets, 8 (13%) gave positive results, while only 12 (3%) shrimp samples were positive for SPT. When the overall results of plate sensitivity were considered, penicillins and tetracyclines were the commonly detected antibiotics in broilers and shrimp.

A Single Plate Assay, which screens antimicrobial residues in animal feed, was also developed and established, where *Bacillus stearothermophilus* var *calidolactis* strain C953 was used as the indicator organism. The routine assay was performed in MHA at pH 7.0. This single plate assay was validated with feed samples spiked with zinc bacitracin, chlortetracycline, streptomycin, furazolidone sulphadiazine, ciprofloxacin and erythromycin. Accordingly Minimum Detectable Concentrations (MDC) were established for different antimicrobials.

In the single plate assay for animal feed, the best sensitivity (0.0025 g/kg) was given with erythromycin while flavomycin was not detectable. All the feed ingredients showed negative results, excluding the possibility of having false positive results. This single plate assay for feed is suitable as a preliminary screening test to detect the presence of inhibitory antimicrobial substances in animal feed and provides with a simple and rapid, multi-analytic screening test.

The validation results and the results of survey samples justify the suitability of modified EU-SPT in screening antimicrobial residues in broilers and shrimp. The modified EU-SPT and Single Plate Assay for feed are unable to quantify or confirm the inhibitory antimicrobial agent. The detection of residues at the phase of screening necessitates the establishment of a secondary screening procedure as Thin Layer Chromatography (TLC) method and confirmatory method as High Performance Liquid Chromatography (HPLC), to confirm and identify the detected residue.

**Key words: Residue screening, Microbial Inhibitory Test, Testing feed, Residues in poultry, Residues in shrimp**