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Evaluating the Applicability of the Modified Four-Plate Test on the Determination of Antimicrobial Agent Residues in Pork

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ABSTRACT

The antibiotic residues in 1,022 pork carcass samples, which were collected from 19 areas in Taipei, Taichung and Kaohsiung counties between January and June, 1997, were systematically investigated using a modified four-plate test (MFPT). Four culture media inoculated with 3 strains of bacteria were used; two were inoculated with *Bacillus subtilis* at pH 6.0 and pH 8.0, and the third and fourth were inoculated with *Bacillus cereus* and *Sarcina lutea*, respectively. The identification and quantification of tetracycline residues in the raw meat was performed by bioassay and bioautography. Streptomycin and chloramphenicol in pork carcasses were screened by ELISA and further quantified using the HPLC method. Results showed that using the CNS 5916 method, only 1 out of 1,022 test samples (0.1%) was detected to contain an antimicrobial agent. While using the MFPT method, 4.11% and 0.64% test samples were found to contain tetracycline and streptomycin residues, respectively. No chloramphenicol residue was detected in any tested samples. It was concluded that the MFPT method is practical, sensitive, less expensive, and simple as compared to the CNS 5916 and FPT methods, which are routinely used in Taiwan and the European Community, respectively. MFTP is therefore recommended as an official method to assay the antimicrobial agents in foods.

Key words: **Taiwan area, pork, antimicrobial agent residues, modified four-plate test, tetracyclines, streptomycin, chloramphenicol.**

INTRODUCTION

Pork is the major animal consumed in Taiwan. It is an important source of animal protein. Taiwan is located in a subtropical zone with hot and humid island weather. To improve health and safety, pigs are usually dosed with antibiotics to promote their growth and prevent or cure disease. However, the rules of use of antimicrobial agent must be obeyed to prevent antibiotics residue in carcasses. In this study, a method referring to those adopted by Europe and Japan was established to routinely analyze and monitor antibiotics in domestic as well as imported pork products.

The bioassay method adopted by Europe and Japan is only used for screening antimicrobial agents. Test samples with positive reaction have to be further identified and quantified for the antimicrobial agents. There are two Chinese National Standard (CNS) methods currently used to test the

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antibiotic substances in fresh meat. They are CNS 5916 entitled "Methods of Test for the Residual Antibiotic Substances in Fresh Meat" and CNS 12322 entitled "Method of Test for Residual Tetracyclines in Meat and Meat Products"[2, 3]. The CNS 5916 is a bioassay method in which a single strain of *Bacillus subtilis* (*B. subtilis*) BGA and a single microbial culture medium at pH 6.0 are used. This method, however, is not sensitive enough to differentiate the residual antimicrobial agents in meat. In Europe, a Four-Plate Test (FPT)[4] is used to assay multiple antibiotic substances as listed in Table 1. However, a culture medium at pH 7.2 containing *B. subtilis* (B-7.2 agar) lacks sensitivity in detecting sulfa drugs, making FPT impractical in use of antibiotic substances assay.

A simplified method for testing residual antibacterial substance is used in Japan[5]. This method involves 3 microbial strains and 4 culture media as follows: *B. subtilis* at ATCC 6633 culture medium at either pH 6.0 or 7.2, *Micrococcus luteus* (*M. luteus*) at culture medium pH 8.0, and *Bacillus cereus* var. *mycoides* (*B. cereus*) at culture medium ATCC 11778. The culture medium ATCC 11778 is sensitive in detection of tetracyclines; however, by using this method, the test samples need to be homogenized followed by extraction with a citrate acetone buffer. This procedure is time-consuming and is not suitable for large sample preparation. Furthermore, the B-7.2 agar is also used in this method. It is impractical for sulfa substance detection for the same reason mentioned above. In this study, a Modified Four-Plate Test (MFPT) based on the methods of Europe and Japan with some modification was developed to rapidly screen the antibiotic substances in fresh meat. The culture media, microbial strains and the antibiotics to be detected are listed in Table 2.

MATERIALS AND METHODS

I. *Materials*

Pork carcass samples (1,022 in total) were collected from 19 areas in Taiwan between January and June, 1997 as listed in Table 3.

II. *Reagents and Devices*

(I) *Standard Strains*

Table 1. Cultural media, indicatory bacteria and their inferred antibiotics of the FPT used in Europe

Medium	Test bacteria	Inferred antibiotics
pH6.0 agar	*Bacillus subtilis* BGA	Penicillin-type Tetracycline-type
pH7.2 agar	*Bacillus subtilis* BGA	Sulfa drugs
PH8.0 agar	*Bacillus subtilis* BGA	Aminoglycoside-type
pH8.0 agar	*Micrococcus lutea*	Penicillin-type Marcolides-type

Table 2. Cultural media, indicatory bacteria and their inferred antibiotics of the MFPT

Medium	Test bacteria	Inferred antibiotics
B-6.0	*Bacillus subtilis* BGA	Penicillin-type Tetracycline-type
B-8.0	*Bacillus subtilis* BGA	Aminoglycoside-type
11778	*Bacillus cereus* var. *mycoides*	Tetracycline-type
9341-8.0	*Sarcina lutea*	β-lactam-type Marcolides-type

Table 3. Number of investigated samples from different Livestock Disease Control Centers in Taiwan

Area	No. of Sample	Area	No. of Sample
Taichung city	10	Taoyuan county	60
Miou-li county	20	Yunlin county	60
Ping Tong county	50	Nan Tou county	60
Hsin Chu county	50	Chiayi city hall	60
Tainan county	60	Tai Tung county	60
Kaohsiung county	60	Hua Lian county	60
Chiayi county	60	Chang Hua county	59
Tainan city	60	Taichung county	60
Peng Hu county	40	Ilan county	73
Taipei county	60		
Total			1022

Sarcina lutea (ATCC 9341) collagen disc (1.8 x 10^8 cell/disc)[6]

Bacillus subtilis BGA collagen disc (1.45 x 10^7 cell/disc)[2]

Bacillus cereus var. *mycoides* (ATCC 11778) collagen disc (3.7 x 10^7 cell/disc)[3]

(II) *Culture Media*

1. *Sarcina lutea* collagen disc was dissolved in 10 mL of sterile saline solution at 48~50˚C. An adequate amount of the above saline solution was then transferred to an Antibiotic Medium 11 (Difco) to make a concentration of 3.6 x 10^5 cell/mL solution. Ten mL of the diluted solution was then transferred to a 9-cm (i.d.) culture medium plate. This culture medium was ready to use when the solidification of collagen was complete.

2. *B. subtilis* BGA collagen disc was dissolved in 10 mL of sterile saline solution at 48~50˚C. An adequate amount of the above saline solution was then transferred to an Antibiotic Medium 1 (Difco) to make a concentration of 1.5 x 10^5 cell/mL solution. Ten mL of the diluted solution was then transferred to a 9-cm (i.d.) culture medium plate. This culture medium was ready to use when the solidification of collagen was complete.

3. *B. subtilis* collagen disc was dissolved in 2 mL of sterile saline solution at 48~50˚C. An adequate amount of the above saline solution was then transferred to an Antibiotic Medium 8 (Difco) to make a concentration of 7.4 x 10^5 cell/mL solution. Ten mL of the diluted solution was then transferred to a 9-cm (i.d.) culture medium plate. This culture medium was ready to use when the solidification of collagen was complete.

(III) *Antibiotic Standards*

1. Oxytetracycline (982.2 μg/mg, titration by National Laboratory of Food and Drugs). The standard was dissolved with a 0.5 mL of 0.1N HCl solution and then diluted to the concentration of 1000 μg/mL as a stock standard solution with a pH 4.5 phosphate buffer solution. The above stock solution was stable at 4˚C for 2 months. Working solutions with concentrations of 0.6, 0.4, 0.2, 0.1 and 0.05 ppm were prepared from stock solution.

2. Chlorotetracycline (936.8 μg/mg, titration by National Laboratory of Food and Drugs). The standard was dissolved with 0.5 mL of 0.1N HCl solution and then diluted with a pH 4.5 phosphate buffer solution to the concentration of 1000 μg/mL as a stock standard solution. The above stock solution was stable at 4˚C for 2 months. The working solutions with concentrations of 0.16, 0.04, 0.02, 0.015 and 0.01 ppm were prepared from stock solution.

3. Chloramphenicol (1000 μg/mg, titration by National Laboratory of Food and Drugs). A stock standard solution in methanol (1000 μg/mL) was

prepared, which was then diluted to a series of concentrations (5000, 1000, 500, 200 and 100 ppb) of working solutions.

4. Antibiotics filter paper disc. gentamicin (GM-10, 10 μg/disc) was obtained from Difco. Oxytetracycline disc (60 ng/disc) was prepared by pipetting 30 μL of 2 ppm oxytetracycline to a sterile filter paper (8-mm i.d., 1.5-mm thickness).

(IV) *Enzyme Conjugate Immunoassay Kit*

1. *Chloramphenicol (CAP) Enzyme Immunoassay (EIA) Kit*

This kit includes the micro-test well, chloramphenicol standard solutions (0.0, 0.31, 0.63, 1.25, 2.5 and 5.0 ppb), working wash solution, CAP-Enzyme Conjugate, TMB substrate solution A and B, Stop solution and PBS. This kit was supplied by the Development Center of Biotechnology (DCB).

2. *Streptomycin EIA Kit*

This kit including the micro-test well, streptomycin standards (0, 5, 20, 80, 320, 1280 ppb), streptomycin-enzyme conjugate, anti-streptomycin antibody concentrate, substrate solution, color-developing solution, stop solution, and a buffer solution was obtained from R-Biopharm GmbH (Germany).

(V) *Reagents*

1. Methanol, ethanol, butanol, hexane, hydrochloride and ethyl acetate were purchased from ALPS (Taiwan). Citric acid, sodium hydroxide, potassium hydrogen phosphate and anhydrous sodium sulfate were reagent grade and purchased from Hayashi Pure Chemical Industries, Ltd.

2. Preparation of XAD-2 resin column. A glass column (300 x 10-mm i.d.) was packed with the XAD-2 resin (Sigma), which was washed with methanol followed by 1N sodium hydroxide, 1N hydrochloride and water prior to packing.

3. Thin Layer Chromatography plate (200 x 200 x 0.1 mm thickness, Art.5552, DC-Alufolien cellulose; Merck) was sprayed with a pH 4.5 buffer solution and dried before use.

4. Developing solvent: butanol: acetic acid: water (10: 1: 2).

(VI) *Equipment and Devices*

1. Stainless drill, 8-mm i.d.
2. Multi-blender (Japan)
3. Centrifuge (KUBOTA 8100)
4. Vacuum rotary evaporator (BÜCHI II)
5. Thermo-incubator (Yamato IN 81)
6. ELISA reader
7. High performance liquid chromatography instrument (Shimadzu LC 10 A)
8. HPLC column (Lichrospher R 100 RP-18e, 25 cm x 4.0 mm i.d., 5 μm, Merck)

III. *Methods*

(I) *MFPT Test*

A pork sample (>4~5 cm^3) was frozen at -20˚C and then drilled into a cylinder shape by using a 8-mm i.d. drill. The cylinder pork sample was then centrally cut into pieces (4-mm i.d.). Four pieces were separately placed on the culture media of B-6.0, B-8.0, 11778 and 9341-8.0 agars with a GM-10 antibiotic filter disc (in 11778 agar, the GM-10 was replaced by a 60 ng OTC filter disc) as a positive control. The above media were cultured at 30 ± 1˚C for 17 ± 1 hr. An inhibition zone greater than 2-mm i.d. was considered as positive; while that less than 2-mm i.d. was determined as negative.

(II) *Test for Detection Sensitivity*

An antibiotics-free pork sample was ground by using a meat chopper, and 40 g of ground pork was then packed into a plastic bag. Three levels (0.06, 1.35 and 2.0 ppm) of oxytetracycline were then spiked into the bag. The spiked samples were well mixed and then stored at -20˚C until use. The MFPT test as listed at III(I) was used to test for the detection sensitivity of oxytetracycline as well as other antibiotics.

(III) *Detection of Oxytetracycline and Chlorotetracycline*

1. Test sample was initially screened using the MFTP method (*B. cereus* ATCC 11778 as a test microorganism). The test sample in MFTP showed an inhibition zone ≧ 0.5 mm i.d. and was further identified and quantified for antibiotics.

2. Identification and quantification of antibiotics. The CNS 12322 method[3] for the detection of residual tetracyclines in meat and meat products was followed. Ten g of pork sample spiked with 30 mL of 0.1% citric acid/ethanol (7: 3) solution was homogenized for 3 min. After centrifugation at 1150 g for 10 min, the supernatant was collected and the residue was then extracted twice with the same solvent. The combined supernatants were mixed with 40 mL of distill water and then centrifuged. The supernatant was evaporated to a volume of about 70 mL under vacuum while keeping at 40˚C. The resulting solution was added with 0.1% citric acid up to a final volume of 100 mL, which was then loaded into a column packed with 10 g XAD-2 resin. The column was rinsed with 50 mL of water before loading sample. After loading the sample solution, the column was washed with 50 ml of distill water and the compound of interest was eluted with 50 mL of methanol. The eluate was then divided into two portions. One was evaporated to dryness and then reconstituted with 5 mL of phosphate buffer solution (pH 4.5) for a quantification test. The quantification test was performed using a cylinder plate test (*B. cereus* ATCC 11778 as a test microorganism). The another portion was evaporated to dryness and then reconstituted with 0.5 mL of methanol for an identification test, which was conducted using a TLC-bioassay[3].

(IV) *Detection of Chloramphenicol*

1. *Chloramphenicol Enzyme-Linked Immunoassay*[7]

Five g pork sample spiked with 20 mL of phosphate buffer solution (pH 6.5) was homogenized and centrifuged at 1150 x g for 10 min. The supernatant (100 μL) and chloramphenicol standard solution (100 μL) were individually placed into a test well where the chloramphenicol enzyme conjugate (100 μL) was then added. The reaction was performed at room temperature for 10 min. After reaction, the liquor in test wells was disposed. The test wells were repeatedly washed with washing solution by 5 times. One hundred μL of the mixture of substrate A and B (1: 1, v/v) was spiked to the test well and allowed to stand at room temperature for 10 min. A stop solution (100 μL) was then added to stop the reaction and the resulting solution was then scanned with an enzyme immunoassay reader at a dual wavelength of 450 nm and 650nm.

2. *HPLC Method*[8]

The pork samples determined as positives (B/B_0<85%) by enzyme-linked immunoassay were further analyzed by HPLC for chloramphenicol quantification. The pork sample (20 g) mixed with ethyl acetate (30 mL) was homogenized for 3 min. After centrifuged at 1150 x g for 10 min, the supernatant was collected. The above homogenization procedure was performed in triplicate. The combined supernatants were evaporated to dryness at 40˚C under vacuum. The residue was then reconstituted with 50 mL of phosphate buffer solution (pH 6.0) and extracted with 50 mL of hexane for 5 min. The aqueous phase was extracted with 50 mL (x2) of ethyl acetate, which was then collected and dehydrated with 10 g anhydrous sodium sulfate. The organic phase was evaporated to dryness and the residue was reconstituted with 1 mL of 50% methanol, which was ready for HPLC analysis. The analytical conditions for HPLC were as follows: mobile phase, 25% acetonitrile; flow rate, 1.0 mL/min; detection at UV 280 nm; chart speed, 0.5 cm/min.

(V) *Streptomycin Enzyme-Linked Immunoassay*[9]

The pork sample (5 g) mixed with 20 mL of PBS-Tween buffer solution was homogenized and then centrifuged at 1150 x g for 10 min. The supernatant (50 μL), streptomycin standard solution (50 μL), enzyme conjugate (50 μL) and antibody solution (50 μL) were stepwise added into the test well. The above mixture was then kept at

Table 4. Number of samples of different inhibiting zones in MFPT of B-6.0 、B-8.0 、11778 and 9341-8.0 agar

Plate test / Inhibiting zone	B-6.0	B-8.0	9341-8.0	11778
>2mm	1	0	1	19
1-2mm	4	2	0	20
<1mm	0	0	0	10

room temperature for 2 hr. The liquor in the test well was disposed of and the test well was repeatedly washed with a washing solution 5 times. After discarding the washing solution, the substrate (50 μL) and developing liquids (50 μL) were added to the same test hole. The above mixture was then kept in the dark at room temperature for 30 min. The reaction was terminated by adding 100 μL of stop solution. The final solution was detected at 450 nm.

RESULTS AND DISCUSSION

I. *The Test for the Antibiotic Residues in Pork*

The test results for the antibiotic residues in 1,022 pork samples collected in 1997 are shown in Table 4. One out of 1,022 test samples (0.1%) was positive as tested using the CNS5916 method. One sample in both B-6.0 and 9341-8.0 agars showed the inhibiting zone greater than 2 mm. By using the MFPT test, 19 samples showed the inhibiting zone greater than 2 mm in the 11778 culture medium. The antibiotic residue was determined to be tetracyclines.

II. *MFPT Used as Screening Test for Detecting Antimicrobial Residues in Pork*

The MFPT test was modified from the FPT method adopted in Europe and a simplified method used in Japan. The MFPT test involved four culture media, *B. subtilis* BGA pH 6.0 and pH 8.0 (B-6.0 and B-8.0), *Sarcina lutea* ATCC 9341 pH 8.0 (9341-8.0), and *B. cereus* ATCC 11778 (11778). A culture medium *B. subtilis* pH 7.2 (B-7.2 agar, the trimethoprim was introduced) was used in our preliminary study. We found it was not sensitive enough to detect sulfa drugs. No inhibiting zone was found in B-7.2 agar where a meat sample with 1 ppm sulfamonomethoxine was placed. Using this medium, the detection sensitivity was not capable of reaching the European standard (MRL 0.1 ppm), but was only able to detect high residue of sulfa drugs in meat products according to research by Okerman[10]. Therefore, the chemical method or an enzyme immunoassay was necessary to replace B-7.2 agar. ATCC 11778 agar, a simplified method adopted in Japan, was used in this study due to its specificity to tetracyclines detection.

Table 5. The detecting sensitivities of various antibiotics by MFPT test

Plate test / Antibiotic	Detecting sensitivities (ppm)			
	B-6.0	B-8.0	9341-8.0	11778
Penicillin	0.05[a]	0.1[a]	0.08 [a]	ND [b]
Ampicillin	0.15	0.18	0.05	ND
Oxytetracycline	1.35	2.0	ND	0.35
Chlorotetracycline	0.1	0.25	ND	0.06
Gentamicin	9.0	1.6	ND	ND
Streptomycin	13.0	1.8	ND	ND
Kanamycin	8.0	2.3	ND	ND
Erythromycin	4.0	1.5	0.45	ND
Tylosin	18.0	12.0	2.0	ND

[a] IU/g. [b] ND: non-detected.

The antibiotic levels that could yield the inhibiting zone > 2 mm ± 0.3 mm in MFPT are listed in Table 5. The detection sensitivities of aminoglycoside including gentamicin, streptomycin, kanamycin and erythromycin were increased by raising the pH from 6.0 to 8.0. The detection sensitivities of marcolide (erythromycin and tylocin) in 9341-8.0 were better than in B-6.0

agar. The sensitivities of erythromycin and tylocin were 0.45 and 2.0 ppm in 9341-8.0 agar and 4.0 and 18.0 ppm in B-6.0 agar, respectively. The culture medium 11778 was highly specific to tetracyclines. When compared to the sensitivities of oxytetracycline and chlorotetracycline (1.35 and 0.1 ppm, respectively) in B-6.0 agar, the 11778 culture medium was capable of detecting the above two tetracyclines as low as 0.35 and 0.06 ppm, respectively. In addition, the 11778 culture medium could be used to screen tetracycline residues in samples; while using B-6.0 agar was not able to differentiate the residues of β-lactams and tetracyclines. The results in Table 5 indicate that the MFPT method could result in better sensitivity and capability in detecting more varieties of antibiotics in meat samples than CNS 5916 (B-6.0 agar).

The MFPT method is simple, fast and capable of detecting antibiotics in fresh meat without using a complicated device. However, the test samples with high water content such as organ meats and egg samples, or meat samples with low water content are not suitable for MFPT assay. A simplified method using citric acid and acetone extraction is recommended as a replacement to the MFPT method in the above cases.

III. *Tetracycline Residues in Pork Samples*

The fortification recoveries of oxytetracycline (OTC), tetracycline and chlorotetracycline (CTC) from pork samples were determined to be 85, 82 and 65%, respectively. Forty-nine out of 1,022 test samples in a 11778 culture medium with inhibiting zone were observed (Table 4). Among them, 7 samples, which appeared in the inhibiting zone <0.5mm was classified as trace residue. Hence, further tetracycline identification was not conducted. The other 42 test samples (4.11%, 42/1022) were determined to be tetracycline-containing samples.

The calibration curves of CTC and OTC are shown in Figure 1 and the results for the tetracycline residue levels in pork samples are presented in Table 6. The results showed 4 test samples con-

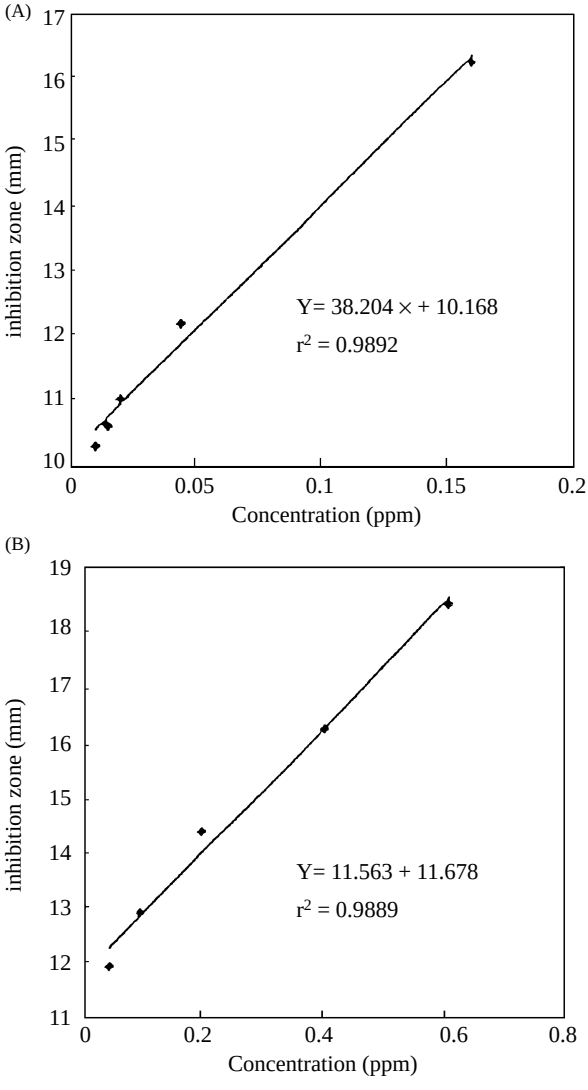

Figure 1. Standard curve of (A) CTC (B) OTC.

Table 6. Number of samples of different tetracyclines residual levels in raw pork samples detected

OTC (ppm)		CTC (ppm)			TC (ppm)	OTC & CTC(ppm)
< 0.1	0.1 ~ 1.0	< 0.04	< 0.1	0.1~ 1.0	< 0.1	< 0.2
11[a]	5	6	10	4	2	4[b]

[a] 11 of 1012 meat samples were positively residual.

[b] 4 of 1012 meat samples contained both OTC and CTC.

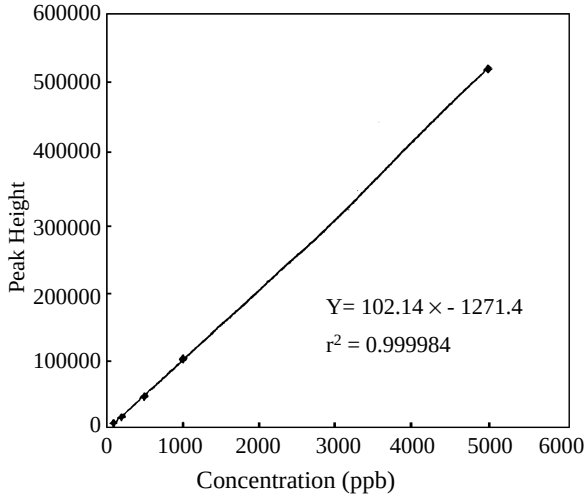

Figure 2. Standard curve of chloramphcnicol.

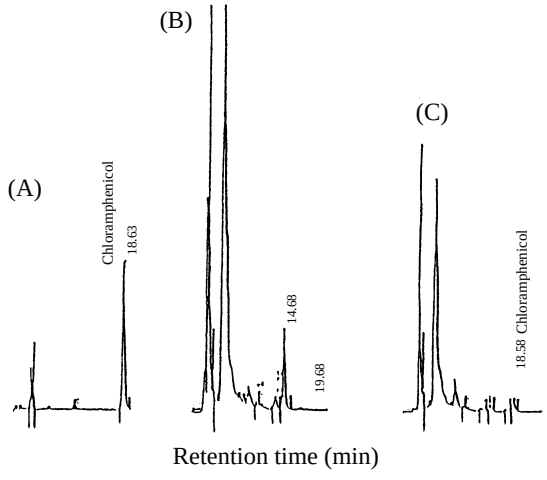

Figure 3. HPLC chromatograms of analyses of (A) chloramphenicol (B) blank and (C) pork containing 0.05 ppm chloramphenicol on Lichrospher C18 column with a mobile phase of water: acetonitrile (75:25) and at a flow rate of 1 mL min^{-1}.

tained both OTC and CTC. Among them, 3 samples were found to retain OTC < 0.1 ppm and CTC < 0.04 ppm; while 1 sample contained 0.2 ppm OTC and 0.09 ppm CTC. The data in this study could be a reference for monitoring the tetracycline residue in pork carcasses in the Taiwan area. The investigation of antibiotic residues in pork carcasses in the fiscal year 1984 was conducted by Lu and Tung[11]. They sampled 120 pork carcasses and assayed using the cylinder plate method. Results showed the residues of tetracycline, penicillin and chloramphenicol were 0, 0.83 and 0.83%, respectively.

Four methods, the cylinder plate[12], enzyme immunoassay, HPLC[13] and CNS 12322[3] were routinely used to assay tetracyclines. In this study, we developed a MFPT method in which a 11778 culture medium was used to effectively detect tetracycline residues in fresh meat. The possible tetracycline-residual samples were further identified and quantified using the CNS 12322 method. Okerman[10] investigated 4,796 fresh meat samples including beef, chicken meat, pork and turkey meat collected from 15 countries in Europe in 1995~1996 using FPT as a screening test. The test samples detected as positive were further assayed for sulfa drugs by TLC, tetracyclines by enzyme immunoassay and macrolide by the receptor test (charm II test). Test results showed 95 samples in *B. subtilis* BGA pH6.0 or pH8.0 agar were positive. Seventy-seven samples were confirmed as tetracycline-residual samples. One contained a sulfa drug and two contained quinolones. The positive samples in pH 6.0 agar were necessary to be screened for detection of tetracyclines using enzyme immunoassay, which was only for identification. The quantification of tetracyclines by using enzyme immunoassay was rarely achieved.

IV. *Chloramphenicol and Streptomycin Residues in Pork*

According to regulation standards for drug residue set by the Department of Health[13], both streptomycin and chloramphenicol should not be detected in meat. Traditional methods for screening could only achieve a ppm level detection for these two antibiotics. In this study, an enzyme immunoassay was used to initially screen the test samples and the samples with positive reaction were further analyzed by HPLC. The results showed no streptomycin residue in 141 test samples, which were randomly sampled from 1,022 pork samples. Two out of 314 test samples (0.64%, 2/314) were detected to be positive for chloramphenicol residue. HPLC analytical results showed the residues were 0.12 and 0.14 ppm. Figure 2 and 3 show the calibration curve and

HPLC chromatogram of chloramphenicol, respectively. The recoveries of chloramphenicol in pork samples with spiking levels of 10, 100, 500 and 1000 ppb were 55, 75.6, 82.1 and 76.4%, respectively. The detection limit was determined to be 5 ppb using the current developed method.

Several methods have been developed to assay chloramphenicol including the cylinder plate method[8], enzyme immunoassay[7] and HPLC method[8]. Enzyme immunoassay is fast and sensitive, and is recommended as a screen test for chloramphenicol detection. The positive samples are further identified and quantified using the HPLC method. Following this procedure, Van de Water[15] reported the detection sensitivities of chloramphenicol in milk and bovine tissue were 1 and 10 ppb, respectively.

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評估以改良式四種培養基檢測法篩檢豬肉抗生物質殘留之可行性

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摘　要

本研究於民國86年1月至6月間，針對台灣區上市前之豬屠體進行抗生素殘留情況之調查；經台北縣、台中縣、高雄縣等19個縣市之家畜疾病防治所檢驗員至肉品市場採豬屠體共1022件，以改良式四種培養基檢測法做初步篩檢肉中抗生物質殘留情形。改良式四種培養基檢測法係檢測生物制菌圈，四種培養基中有二種培養基菌層爲枯草桿菌，其pH分別爲6.0及8.0，第三種培養基菌層爲仙人掌桿菌，第四種培養基菌層爲卵黃色八聯球菌。生豬肉中四環素類抗生素殘留以生物分析法、生物自析鑑別法定量及定性分析。另從1022件樣品中隨機抽樣以酵素免疫分析方法檢測鏈黴素、氯黴素，再以HPLC方法定量。檢測結果豬肉抗生物質陽性率0.1％，四環素類殘留率4.11％，氯黴素殘留率0.64％，鏈黴素殘留率0％。由本研究顯示出改良式四種培養基檢測法爲一種不用昂貴儀器或複雜方法，比目前台灣以CNS5916方法或歐聯新近發展之四種培養基檢測法可檢出更多之抗生素及更靈敏之感度，故建議改良式四種培養基檢測法可取代目前CNS5916方法或歐聯之四種培養基檢測法。

關鍵詞：台灣地區，豬肉，抗生物質殘留，改良式四種培養基檢測法，四環素類抗生素，鏈黴素，氯黴素。