

Development and Evaluation of a Screening Test Kit for Detection of Tetracycline Group Residues in Honey

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ABSTRACT

*Extensive use of antibiotics in bee colonies can cause an accumulation of residues in honey and become a public health hazard. Analysis of antibiotic residues, therefore, is essential for consumer protection purposes. This study developed and evaluated a simple, rapid, and inexpensive screening test kit for detecting tetracycline group residues in honey. A microbial inhibition assay was carried out using spores of *Geobacillus stearothermophilus* (DMST 8041) in optimal medium with bromocresol purple as an indicator. Prepared 0.1 ml of 30% honey solution was incubated at $65 \pm 1^\circ\text{C}$ for 2-3 hours in a water bath. A positive reaction was detected by negligible change of the purple medium, indicating the presence of a substance(s) that can prevent the growth of the test organism. A negative reaction, indicating an absence of antibiotic residue in sufficient quantity to inhibit bacterial growth, showed a complete change of the medium's color, due to bacterial propagation, from purple to yellow.*

Using negative and positive controls (honey free of any residues and honey spiked with varying concentrations of residue, respectively), the screening test kit was 99% accurate. The test kit had a detection limit for tetracycline group residues in honey of 10 $\mu\text{g}/\text{kg}$. The shelf life of the test kit kept refrigerated at 4-8°C was 9 months.

The test kit was then used to test 120 commercially available honey samples from across northern Thailand for tetracycline group residues. All 120 samples tested free of antibiotic residues using the new test kit. Of these samples, 30 were randomly selected and subjected to antibiotic residue test using HPLC technique to validate the results from the new screening test kit. Only one of the samples tested positive (8.85 $\mu\text{g}/\text{kg}$ of chlortetracycline) at a concentration below the 10 $\mu\text{g}/\text{kg}$ detection limit of the test kit.

Keywords: Test kit, Tetracycline, Residues, Honey

INTRODUCTION

Honeybees play a vital role in the environment by pollinating both wildflowers and many agricultural crops as they forage for nectar and pollen, in addition to producing honey and other products. The essential and valuable activities of bees depend upon beekeepers to maintain a healthy population of honeybees because, like other insects and livestock, many microorganisms can infect honeybees. Beekeepers prophylactically administer antibiotics to prevent outbreaks and to treat bacterial pathogens. Tetracyclines, in use since 1967, are still used for the treatment and control of a wide variety of bacterial infections (Dinkov et al., 2005). Currently, the most commonly applied antibiotic against *Mellissococcus pluton* and *Paenibacillus larvae* (causes of the European foulbrood and American foulbrood, respectively) in bees is oxytetracycline; followed by tetracycline, chlortetracycline, and doxycycline (Lehnert and Shimanuki, 1980). This extensive use of antibiotics in honeybee colonies can cause an accumulation of antibiotic residues in honey and can become a public health hazard.

Thailand does not specify recommended maximum residue limits (MRL) for veterinary medicine products in honey, as it has a zero tolerance limit similar to European legislation (Passantino and Russo, 2008). In practice, it effectively prohibits the use of drugs or does not allow for any drug residues in honey products (Suijidta, 2008). This research only investigated the use of tetracycline, chlortetracycline, and oxytetracycline as these drugs are inexpensive and readily available from drugstores or chemical shops for use by beekeepers.

In 2004, the Thai Nestle Company tested honey in Thailand and found chloramphenicol in one of 229 samples and tetracyclines in 30 of 229 samples (Maneetup, 2004). Liawruangrath et al., (2006) found chloramphenicol in 10 of 14 samples of honey tested (0.29-3.26 mg/g) from Chiang Mai Province but found no tetracyclines using High Performance Liquid Chromatography (HPLC). Pathomchai and Sujaritpun (2007) found chloramphenicol in three out of six samples of honey from Chiang Mai Province (2.49-10.69 µg/kg) but again no tetracyclines as analyzed by HPLC. Pochalearn (2007) found tetracycline group residues in 2 out of 267 samples of honey from Chiang Mai Province using Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) technique. (Note: Chloramphenicol is no longer available for purchase/use in beekeeping in Thailand.)

In northern Thailand, plantations such as longan, forest flower, and bitter bush occupy the majority of orchards. Northern Thailand has many beekeepers and honey factories and is an important source of honey exports. Thai beekeepers generally use antibiotics to control microbial infections in bees because of its convenience and quick results. This may lead to significant antibiotic residues in honey and other bee products. A screening test for antibiotic residues in honey is, therefore, required to protect consumers.

To address this issue, this study has aimed to develop an effective and commercially applicable microbial inhibition assay screening test kit for detecting tetracycline group residues. To do so, this study will seek to develop a test kit based on using a microbial inhibition assay as it has several advantages—

it is inexpensive, easy to use, adaptable to screening large numbers of samples, and can detect a wide variety of antibiotics (and/or chemicals toxic to bacteria). However, such a test kit cannot be used to identify the specific antibiotic residue present, produces qualitative (presence of residuals or not) rather than quantitative results (specific concentration levels), and requires a few hours (2-3) before results are available. For positive results, a validated HPLC or LC-MS/MS method must subsequently be used to confirm and quantify the specific antibiotic presence. The test kit developed here will then be evaluated for its effectiveness in detecting tetracycline group residues and compared against and validated using the HPLC technique.

MATERIALS AND METHODS

A simple screening test kit was developed at the Central Laboratory of the Veterinary Medicine Faculty, Chiang Mai University based on the microbial inhibition assay method. To evaluate this test kit, three studies were subsequently carried out. The first study determined the detection limit of the screening kit and validated results using the HPLC method. The second study investigated the shelf life of the screening kit. The final study used the screening kit to test for tetracycline group residues in commercially available honey samples from private beekeepers and markets in northern Thailand.

Test kit development

The screening test kit developed here is an ampoule, containing an agar medium, imbedded spores of *Geobacillus stearothermophilus* (DMST 8041) and a color indicator. The screening test combines the principle of an agar diffusion test with a color change indicator. Bromocresol purple is a pH indicator that detects changes in acidic conditions, changing color over a pH range of 5.2-6.8. In the case of active metabolism of the included microorganism, acid is produced and the bromocresol changes color from purple to yellow in response to the changing pH levels. The optimum functional temperature of the included bacteria is 60-65°C and the optimum pH for growth is 7.0. The spores will not develop if the test kit is stored at temperatures below 43°C. The test kit should be stored at 4-8°C, not frozen, and kept away from direct sunlight.

When using the test kit at optimal growth conditions, if microorganism growth is inhibited by the presence of an antibiotic at or above the detection limit, the test will remain purple. The color of all test sample ampoules is read at the moment that the negative control sample changes color from purple to yellow. The following steps are required to prepare the microbial inhibition assay screening test kit for use:

Preparation of blank honey. Longan honey was collected from a typical bee farm that practiced good beekeeping in Chiang Mai, Thailand during February-April 2010. The honey was prepared to eliminate its osmotic or inhibine effect on microbial assay by dilution at 10-40% with sterile solutions at pH 5 and heating

at 30, 65, 85, and 95°C for 5 minutes. The prepared samples were kept at 4-8°C in a refrigerator. The samples were examined for their microbiological, chemical and physical properties, as well as the presence of any tetracycline group residues. If the samples contained any chemical residues, they were rejected for use with the negative and positive controls.

Preparation of bacteria. *Geobacillus stearothermophilus* (DMST 8041) was acquired from the Microbiological Resources Center of Thailand, Institute of Scientific and Technological Research, Thailand. Prior to use, the cells were streaked on nutrient agar plates and incubated at 65°C for 24 hours under aerobic conditions. After incubation, they were propagated in nutrient broth at 65°C for 72 hours. The cells were then harvested by cool centrifugation at 2,192 g for 20 minutes at 25°C and washed three times with sterile normal saline and then incubated at 95°C for 20 minutes (Holt et al., 1994). The final cells were harvested by refrigerated centrifugation at 2,192 g for 20 minutes at 4°C. The harvested bacteria cells were mixed with normal saline to prepare the cell suspension at 10^8 - 10^9 cfu/ml.

Production of test kit. The medium (yeast extract 0.25%, peptone 0.5%, glucose 0.1%, polysorbate 0.1%, L-cystein 0.01%, agar 1.5%, starch 0.8%, NaCl 0.05%, and bromocresol purple 0.006%) was mixed with deionized water and brought to 100 ml volume and sterilized at 121°C and 15 psi pressure for 15 minutes. The medium (0.4 ml) was then dropped in a polypropylene tube with cap and 0.1 ml of the bacteria suspension was added.

Production of negative and positive controls. Negative controls were prepared by adding 0.1 ml of the prepared blank honey samples. Positive controls were prepared by adding 10-1000 µg/kg antibiotics in the prepared blank honey samples at 30, 65, 85 and 95°C for 5 minutes. Antibiotic standards of HPLC grade (Sigma Chemical Co., USA) were tetracycline (98% purity), oxytetracycline ($\geq 97\%$ purity) and chlortetracycline ($\geq 78\%$ purity).

Screening of the controls. Negative and positive controls were incubated at 65°C for 120-180 minutes. The test was read as negative or positive by the change of color. In the absence of antimicrobial substances, the entire solid medium turned yellow. The incubation time was recorded when the negative controls turned completely yellow. The medium remained purple in the presence of sufficient concentrations of antibiotics. At intermediate concentrations of antibiotic, the solid medium turned partly yellow. The medium changed color from the bottom up. Positive results were identified if the bottom three-fourths part of the ampoule turned yellow. The experiment was carried out using a complete randomized design (CRD) with five replications.

Screening test validation and result comparison with HPLC technique

The results obtained from the antibiotic residues screening test kit using optimal formula and test conditions was validated and compared against the HPLC technique (Pena et al., 2005). Analysis of the tests was validated based on sensitivity, specificity and accuracy of the test (Smith, 2006).

Shelf life of screening test kit

The test kit with optimal components for the microbial assay was stored at 4-8°C. Effectiveness of the test kit was evaluated during the storage from 1 to 270 days. The assay was conducted using diluted honey without antibiotic and diluted honey with antibiotic at 10-100 µg/kg (tetracycline, chlortetracycline and oxytetracycline concentrations) in optimal diluted solution. The experiment was conducted using a complete randomized design with five replications.

Using the test kit to screen commercially available honey from northern Thailand

Honey samples from different geographic locations in northern Thailand were obtained from private beekeepers (76 samples) and local food stores (44 samples) during June-August 2010. A total of 120 honey samples were examined for their physical properties as follows: color quality by using colorimeter (Minolta, Chroma Meter CR 300, Japan), pH by using pH meter (Cyberscan, model 310, Singapore), total soluble solid content by using hand refractometer (ATAGO, model N-3E, Japan) and water activity (aw) by using water activity analyzer (AQUA. LAB, model Series 3TE, USA). All tests were done in triplicate except color quality, which was done in ten replicates, with appropriate controls at each step. The screening test kit was then used to test for the presence of tetracycline group residues in the 120 honey samples. For validity, thirty of the samples were randomly selected by Win Episcopo (Thrusfield et al., 2001) and subjected to tetracycline group residues analysis by HPLC technique.

Sample preparation and HPLC analysis of antibiotics from commercial honey. The sample of honey (10-15 g) was weighed into a polypropylene tube and dissolved in 50 ml of 0.1 M Na₂ EDTA-McIlvaine buffer (pH 4.0). The sample solution was shaken for 5 minutes and centrifuged at 2,850 g for 15 minutes. After filtration, it was loaded on C 18 Clean-up (VertiPak™) 6 cm³ (500 mg) cartridge previously conditioned with 5 ml of methanol and 5 ml of water. The cartridge containing the sample was then washed with 5 ml of methanol. Then, the tetracyclines were eluted with 5 ml of oxalic acid (1 M)/acetonitrile (80/20). The final eluate was filtered through a 0.45 µm cellulose acetate membrane and stirred in vortex before injecting into the HPLC system.

HPLC analyses of tetracycline group residues was applied according to Geertsen and Pedersen (2009) with a Diode Array Detector and Zorbax Eclipse Separation Column (15 cm x 4.6 mm, 5 µm) at a light wavelength of 356 nm with C 18 Guard Column. The mobile phase used acetonitrile:methanol:oxalic acid 0.01M (10:10:80) at a flow rate of 1.0 ml/minutes, injection volume of 100

µl and column temperature of 30°C. The calibration graphs were constructed with standard solution concentrations ranging from 13-74 µg/kg. The linearities were evaluated by linear regression analysis, which was calculated using least square regression. All the solvents used (acetonitrile, methanol, ethyl acetate and water) were of HPLC grade. Other reagents were of analytical grade and purchased from Merck Ltd., Germany.

Statistical evaluation. All results, where possible, were statistically analyzed by analysis of variance using Microsoft Office Excel 2003. If a significant primary effect was detected, the means are followed by $\pm x$, where x refers to the standard deviation. The predetermined acceptable level of probability was 5% ($p \leq 0.05$) for all comparisons (e.g., t -tests, one-way analysis of variance).

RESULTS

Quality of blank longan honey

The longan honey sample had L^* (luminosity), a^* (redness) and b^* (yellowness) values of 37.27 ± 0.47 , 6.22 ± 0.05 and 23.21 ± 0.86 . It had pH, total soluble solids and water activity (a_w) of 3.97, 80.6°Brix and 0.55, respectively. Yeast and mold and total bacteria counts were lower than 10 cfu/g and 100 cfu/g, respectively. The longan honey samples did not contain any tetracycline group residues or any chemical residues.

Negative control

Using the negative control, the screening test kit turned completely yellow after incubation for 150 minutes. Honey diluted at 10-30% concentrations and incubated at 30, 65, 85 and 95°C for 5 minutes all resulted in the test kits turning a completely clear yellow color. At 40% concentration and incubated at 30, 65, 85 and 95°C for 5 minutes, the test kits turned an incomplete yellow color (purple in the upper and yellow in the lower portion of the test kit) after incubating for 150 and 180 minutes because of the high osmotic pressure of honey, which can inhibit bacterial growth.

Positive control

The various samples of positive control (10-1000 µg/kg antibiotic in honey) were tested in the screening kit for 150 minutes. Before testing, the entire 18.5 mm assay column of media was purple. The more the bacteria were able to flourish (indicating low or non-existent levels of antibiotic residue), the more the column changed to yellow from the bottom up. The measurements in Table 1 indicate the mm of yellow produced.

The honey samples mixed with 10 µg/kg oxytetracycline, tetracycline and chlortetracycline in media turned completely yellow at a 10% honey concentration and almost completely yellow at a 20% honey concentration producing false negatives. The test kit was unable to reliably detect the presence of antibiotic residue in the positive control samples with very low residue concentrations

(10 µg/kg) coupled with low honey concentrations – the combination of which created very low residue concentrations (shaded area in Table 1).

For higher residue concentrations and/or higher honey concentrations, the color purple remained in at least a portion of the column, indicating the screening test was able to reliably detect the presence of residue in the positive controls (unshaded area in Table 1).

There was no significant difference in the height of yellow color part, regardless of the antibiotic, between all tested samples at the same concentration.

Table 1. Height (mm) of yellow color formation on positive reaction at various honey concentrations and incubation temperatures.

Concentration and condition of honey sample (all 5 min)	Oxytetracycline			Tetracycline			Chlortetracycline		
	1000 µg/kg	100 µg/kg	10 µg/kg	1000 µg/kg	100 µg/kg	10 µg/kg	1000 µg/kg	100 µg/kg	10 µg/kg
10%, 30°C	4.50	8.70	18.50	4.70	8.00	18.50	7.00	10.00	18.50
10%, 65°C	4.30	8.50	18.50	5.00	8.00	18.50	6.30	10.00	18.50
10%, 85°C	5.00	8.50	18.50	5.10	8.00	18.50	7.00	10.00	18.50
10%, 95°C	5.50	8.50	18.50	5.00	8.00	18.50	7.50	10.00	18.50
20%, 30°C	4.00	8.50	16.00	4.50	7.50	15.50	5.60	9.50	16.50
20%, 65°C	4.00	8.00	15.50	4.50	7.00	15.00	4.50	9.00	16.00
20%, 85°C	5.00	8.00	15.50	4.50	7.00	15.00	5.00	9.00	16.00
20%, 95°C	5.00	8.00	15.00	4.00	7.00	15.00	4.70	8.50	15.00
30%, 30°C	4.50	8.00	13.00	4.00	7.00	13.00	4.50	10.00	13.00
30%, 65°C	5.00	9.00	12.00	4.00	7.50	12.50	5.10	9.00	13.50
30%, 85°C	4.50	8.50	12.00	4.00	7.00	12.50	5.00	9.00	13.00
30%, 95°C	4.10	8.00	13.50	3.60	7.00	12.00	4.50	8.50	13.00
40%, 30°C	4.00	8.00	12.50	3.50	6.70	11.50	4.00	8.00	12.50
40%, 65°C	4.00	8.00	12.00	3.30	6.50	11.00	4.50	8.00	12.00
40%, 85°C	4.00	8.00	12.00	3.00	6.50	11.00	3.70	8.00	12.00
40%, 95°C	4.00	8.00	12.50	3.00	6.50	10.00	4.00	8.00	12.00

Note: Shaded area represents false negatives. Antibiotic residues are present at a concentration undetectable by the screening test.

When the positive control (honey spiked with 100 and 10 µg/kg antibiotics) was further analyzed by HPLC technique, the detected concentration of antibiotics was lower than the spiked concentration (Table 2). It has been reported that the tetracycline group is more stable in acidic conditions (approximately pH 4) than at normal to alkaline conditions (Moreno-Cerezo et al., 2001; Wu and Fassihi, 2005). Antibiotic standard solution and spiked honey samples were prepared fresh daily and kept below -20°C until analysis. The decrease in concentration was not attributed to the storage method as the samples were aseptically collected and stored in sterile bottles with screwed caps.

Recoveries of the antibiotics were different depending on the compound and substrate used with the HPLC technique. The mean recoveries at 10 and 100 µg/kg antibiotic concentrations were 88.8% and 97.2% for tetracycline, 95.4% and 90.3% for oxytetracycline and 81.2% and 89.3% for chlortetracycline, respectively.

Tetracyclines were found to have good linearities between their concentration and peak area responses, ranging from 13-74 $\mu\text{g}/\text{kg}$ with correlation coefficient (r^2) more than 0.999.

Table 2. Values of spiked honey samples with antibiotic by HPLC technique.

Concentrations of antibiotics	Oxytetracycline		Tetracycline		Chlortetracycline	
	100 $\mu\text{g}/\text{kg}$	10 $\mu\text{g}/\text{kg}$	100 $\mu\text{g}/\text{kg}$	10 $\mu\text{g}/\text{kg}$	100 $\mu\text{g}/\text{kg}$	10 $\mu\text{g}/\text{kg}$
HPLC technique	90.29 \pm 1.25	9.54 \pm 1.15	97.18 \pm 0.15	8.87 \pm 0.24	89.27 \pm 1.05	8.11 \pm 0.92

Note: Values were the means of three replications \pm Standard deviation.

Screening kit validity

The clinical sensitivity, specificity and accuracy of the assays were determined by using two-by-two contingency tables (Table 3). For validity, positive controls (honey with antibiotics present – oxytetracycline, tetracycline and chlortetracycline) were tested 100 times and negative controls (honey with no antibiotics) were tested 100 times. The results showed:

- Sensitivity = $(a/a+c) \times 100\% = (100/100+0) \times 100\% = 100\%$
- Specificity = $(d/b+d) \times 100\% = (98/2+98) \times 100\% = 98\%$
- Accuracy of the test = $(a+d/n) \times 100\% = (100+98/200) \times 100\% = 99\%$

Table 3. Validity of screening test kit.

	Honey with antibiotic	Honey without antibiotic	
Result of positive	a (100)	b (2)	a+b (100+2)
Result of negative	c (0)	d (98)	c+d (0+98)
	a+c (100+0)	b+d (2+98)	n (200)

Detection limits of screening kit

The Limits of Detection (LOD) by HPLC technique for tetracycline, chlortetracycline and oxytetracycline in honey are lower than the minimum detection limits of the screening test kit (Table 4).

Table 4. Detection limits of the screening test kit compared to HPLC technique.

Antibiotic	Detection limit of test kit	Detection limit of HPLC
Tetracycline	10 $\mu\text{g}/\text{kg}$	1.048 $\mu\text{g}/\text{kg}$
Oxytetracycline	10 $\mu\text{g}/\text{kg}$	4.462 $\mu\text{g}/\text{kg}$
Chlortetracycline	10 $\mu\text{g}/\text{kg}$	2.811 $\mu\text{g}/\text{kg}$

Shelf life of screening kit

From the study of the effect of storage time on the effectiveness of the screening test kit, after storing the kit for 1 day at 4-8°C, negative control honey turned the kit completely yellow after 150 minutes. After storage for 270 days at 4-8°C, negative control honey turned the kit completely yellow after 180 minutes incubation time. Honey samples mixed with 10 $\mu\text{g}/\text{kg}$ oxytetracycline, tetracycline and chlortetracycline showed complete, almost complete, and partial inhibition

of bacteria growth in test kits with 1-day shelf life at 10, 20 and 30-40% honey concentrations, respectively. The test kit with 270-days shelf life did not show microbial growth inhibition when tested with the sample containing 10% honey concentration and 10 µg/kg oxytetracyclin, but did with the 30% honey concentration. Given this, a 30% honey concentration was the optimum dilution for use with the new antibiotic residual screening test kit (Table 5).

Table 5. Mean values for the height (mm) of yellow color on shelf life of antibiotic residues test kit with effect of 10-40% concentration in honey on a positive reaction.

Honey concentration	Oxytetracycline				Tetracycline				Chlortetracycline			
	100 µg/kg		10 µg/kg		100 µg/kg		10 µg/kg		100 µg/kg		10 µg/kg	
	1 day	270 day	1 day	270 day	1 day	270 day	1 day	270 day	1 day	270 day	1 day	270 day
10%	8.7	7.2	18.5	18.5	8.0	6.5	18.5	8.7	10.0	7.5	18.5	12.5
20%	9.0	6.0	18.0	9.0	7.0	5.2	16.5	8.0	9.5	7.5	18.0	8.0
30%	8.0	6.0	13.0	8.0	7.0	5.2	13.0	8.0	9.0	7.0	13.0	8.0
40%	8.0	6.5	12.5	8.5	6.7	5.2	11.5	7.0	8.0	6.5	12.5	7.5

Using the test kit to screen commercially-available honey samples

The analysis of the 120 honey samples collected from bee farms, honey factories, and markets revealed mean values of L* (luminosity), a* (redness) and b* (yellowness) values of 48.30±0.23, 3.31±0.45 and 32.67±0.23, respectively. The samples had mean values of pH, total soluble solids and water activity (aw) of 3.77±0.30, 80.41±1.15°Brix and 0.59±0.02, respectively.

Using the new screening test kit, no tetracycline group residues were detected in any of the 120 samples. From the 120 samples, thirty samples from a variety of flower sources (longan [15], bitter bush [4], sunflower [3], forest flower [2], unknown [2], rambutan [1], sesame [1], lychee [1], acacia [1]) were randomly selected for validation by HPLC technique. Only one of the 30 (the lychee honey sample) tested positive for chlortetracycline residue (8.85 µg/kg).

DISCUSSION

In positive controls, containing 10-1,000 µg/kg of antibiotic, the solid medium turned partly yellow when the samples with 30% honey concentration incubated at 30, 65, 85, and 95°C C for 5 minutes were tested, and bacteria growth was not different. For use in the new screening test kit, it was found that diluted honey at 30% concentration was the optimum concentration for preparation of honey samples with incubation at 65°C for 2-3 hours in a water bath to destroy natural inhibines. But a 40% concentration of honey cannot be used because of its high osmotic pressure, which can naturally inhibit the growth of bacteria.

The sensitivity of the test kit was 100% and the accuracy of the test was 99% from the preparation with or without antibiotic samples within the concen-

tration range that the antibiotic could be detected. Specificity of the test kit was 98%.

The test kit had a detection limit for tetracycline group residues of 10 µg/kg. The tetracycline group residue concentrations in the honey samples detected by the screening test kit were low and not likely to cause any acute health effects although chronic effects cannot be ruled out. However, even very low levels of antibiotics could over time lead to antibiotic resistance in pathogenic bacteria making their treatment difficult.

As tested, the usable shelf life of the test kit was at least nine months, assuming optimal storage conditions, including immediate and continual storage in refrigerated conditions. After nine months, the bacteria's viability in the nutritional medium decreases, even under optimal conditions, so it is not recommended to use the kit after this period.

Recently, a commercial screening test kit has become available (Premi®test), which is also based on the inhibition of the growth of *Geobacillus stearothermophilus*, the same microbial used in this research (Stead et al., 2004). However, Premi®test has detection limits for tetracycline (50 µg/kg), oxytetracycline (75 µg/kg) and chlortetracycline (80 µg/kg) that are higher than the screening test kit developed here. In addition, to use the Premi®test kit, the honey sample has to be heated at 45°C for 30 minutes or use the acetone extraction method, followed by a two-step incubation (first = 10 minutes at 80°C; second = 3 hours at 64°C). These steps are in contrast to the newly developed screening test kit evaluated here that only requires dilution of the honey sample and one-step incubation (2-3 hours at 65±1°C). The screening test kit developed here is easier to use and has a lower LOD.

Testing commercially available honey from northern Thailand found virtually no evidence of tetracycline group residues. Of the 120 samples tested with the new screening kit, all tested negative for the presence of antibiotic residues. Only when 30 randomly selected samples were further tested for validation by HPLC technique, did one reveal any residue (8.85 µg/kg of chlor-tetracycline) at a concentration below the limits of detection (10 µg/kg) of the new microbial inhibition assay screening test kit.

CONCLUSION

This research has developed a screening test kit in a micro vial polypropylene tube for antibiotic residue detection in honey. Even though the screening test kit developed in this research produces qualitative results only, it is useful for rapid screening of honey for antibiotic residual contamination. The test kit is able to detect antibiotic residues at very low concentrations (10 µg/kg) and has a useable shelf life of nine months. Moreover, the test kit is inexpensive and simple to use. Beekeepers and general staff at honey collection centers can follow simple application instructions for testing for antibiotic residues. If required, the quantity and type of residue can be analysed and confirmed by commercial and more sophisticated techniques such as HPLC and LC-MS/MS technique.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the financial support of the Faculty of Veterinary Medicine, Chiang Mai University.

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