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# Development and Fast Screening of Salbutamol Residues in Swine Serum by an Enzyme-Linked Immunosorbent Assay in Taiwan

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The analysis of salbutamol in swine serum is the more practical basis for large scale surveillance programs in Taiwan. Objectives of the study were to develop a new assay and to compare with a commercially available kit in field test screens. A simple and reliable enzyme-linked immunosorbent assay (ELISA) to monitor the presence of $\beta$-agonist, salbutamol, in 1,358 field samples of swine serum that were collected from local meat markets was described. The method proved to be suitable and sensitive for the detection of $\beta$-agonist residues caused by growth promoting dosage. The limit of detection of the developed ELISA directly performed on diluted serum was 0.25 ng/mL. The application and the results of two ELISA kits (homemade and commercially available) for the screening of salbutamol were presented. For further confirmation, all samples that showed to be ELISA positive for salbutamol residues were analyzed by GC-MS. Adopting 1 ng/mL salbutamol as a cutoff value, the commercial $\beta$-agonist ELISA had a sensitivity of 89.2% and a specificity of 86.7% versus GC-MS at a cutoff of 1 ng/mL. The homemade salbutamol ELISA had a sensitivity of 81.1% and a specificity of 98.6% and gave a low proportion of false-positive rate results. The reliability of the developed kit in terms of the percentage of false-positive rate results is evaluated. In conclusion, a sensitive, specific salbutamol ELISA has been developed that could serve as a rapid screening assay, and the detection of positive samples at the place of sampling can result in more effective control of the illegal use of $\beta$-agonists.

**KEYWORDS: Enzymatic methods; immunoassay; $\beta$-agonists; salbutamol; swine serum; enzyme-linked immunosorbent assay (ELISA); residues**

## INTRODUCTION

Within the $\beta$-agonist drugs illegally used as repartitioning agents in food producing animals, salbutamol and clenbuterol are repeatedly discovered during routine control by national authorities. It has been apparent that the drug shows a repartitioning activity resulting in an increase in muscle tissue accretion and reduced fat deposition making it an important veterinary drug as a growth promoter and fattening agent in cattle, sheep, pigs, and poultry (*1, 2*). Even though banned through the European Union for mass therapy as bronchodilators (*3*), they are still used to improve growth performance, carcass quality, and productivity at dosage levels ≥10-fold than the therapeutic dose.

Residues of these $\beta$-agonists could present a potential risk for public health safety. Effective surveillance for the illicit use of $\beta$-agonists requires sensitive and satisfactory analytical techniques for their detection in serum or tissues from treated animals, and several reports on improved techniques for salbutamol detection have been published (*4–13*).

The screening procedure is a powerful means to detect and tract the illegal use of salbutamol, clenbuterol, and terbutaline in animal production, and several conventional immunoassays methods are commonly used (*14–18*). Most commercially available competitive enzyme-linked immunosorbent assay (ELISA) kits are specific to $\beta$-agonist clenbuterol. An immu-

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**Table 1.** Cross-Reactivities of Antibody with Different Compounds (Manufacturer's Data)

| | cross reactivity (%) | | |
|---|---|---|---|
| compound | Randox (UK) | r-Biopharm (Germany) | Tecna (Italy) |
| clenbuterol | 100 | 100 | 100 |
| carbuterol | 90 | 4.5 | |
| salbutamol | 86 | 11 | 20 |
| methyl-clenbuterol | 75 | | |
| brombuterol | 53 | | |
| terbutaline | 50 | 10 | |
| mabuterol | 45 | | |
| pirbuterol | 25 | 0.9 | |
| mapenterol | 32 | | |
| cimaterol | 10 | 5.5 | |
| ractopamine | <0.2 | | |
| feneterol | <0.2 | | |
| mubaterol | | 71 | 60 |
| isoproterenol | | 4 | |
| orcitrenalin | | 1.1 | |
| adrenaline | | <0.01 | |
| Paylean | | | |

**Table 2.** Kits Employed to Quantitatively Measure the Level of $\beta$-Agonists in Different Samples (Manufacturer's Data)

| sample available | | |
|---|---|---|
| Randox (UK) | r-Biopharm (Germany) | Tecna (Italy) |
| urine | urine | urine |
| muscle | tissue | serum |
| kidney | retina | feed |
| liver | feed | milk |
| retina | | liver |

noassay obtained from Tecna of Italy, shows a 20% cross-reactivity toward salbutamol. With the assay RIDASCREEN manufactured by R-Biopharm of Germany, clenbuterol shows an 11% cross-reactivity toward salbutamol. The cross-reactivities of the antibody with the different compounds are given in **Table 1** (manufacturer's data). However, the analysis of salbutamol in swine serum is the more practical basis for large scale surveillance programs in Taiwan. The commercially available kits, which are specific to clenbuterol, would cause salbutamol false positive test results, and the potentially positive samples would then require confirmation. These techniques rely on expensive instruments operated by well trained analysts and prior preparation of analytes, which is time-consuming, and therefore not ideal for screening large number of samples. The kits that may be employed to quantitatively measure the levels of $\beta$-agonists in different sample matrices are presented in **Table 2**. We evaluated the performance of such a $\beta$-agonist ELISA kit from Randox of the UK. It is recommended for the detection of the following (% of cross-reactivity): clenbuterol (100%), salbutamol (86%), terbutaline (50%), and so forth, even though this kit does not quantitatively measure serum samples.

For these reasons, the serum sample and extraction-free method is to be preferred. We thus generated polyclonal antibodies against salbutamol and further developed a quantitative enzyme immunoassay using a salbutamol substrate for the measurement of horseradish peroxidase (HRP) labeled salbutamol activity. Our work shows the advantage of good specificity, high sensitivity, and convenience in rapid screening of salbutamol residues.

To detect the use of illegal bronchodilator drugs and to prevent the use of growth promoting dosage, veterinarians and the Bureau of Animal and Plant Health Inspection and Quarantine (BAPHIQ) in Taiwan are required to develop extensive monitoring and screening programs for the residues of these drugs in the serum, feed, meat, and meat-related products destined for human consumption. In the testing for veterinary drug residues, there usually is a two-step process. The first step is a screening test which is low in cost, high-volume, and rapid. The negative samples mean that no further action is needed; otherwise, the potentially positive samples require confirmation. The main analytical performance of the developed method is reported herein, and its applicability to direct analysis of $\beta$-agonist in swine serum sample is discussed, so as to make the immunoassay suitable for high throughput screening.

## MATERIALS AND METHODS

**Reagents.** Salbutamol, salbutamol hemisulfate salt, clenbuterol hydrochloride, terbutaline hemisulfate salt, isoproterenol hydrochloride, succinic anhydride, *N*-hydroxysuccinimide, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride, ovalbumin (OVA), bovine serum albumin (BSA), Freund's complete/incomplete adjuvant, and *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) were commercially available from Sigma (St. Louis, MO, USA). Salbutamol, VETRANAL, analytical standard was from Riedel-de Haën (Germany), and Salbutamol-*tert*-butyl-$d_9$ was from Aldrich (Wisconsin, USA). Paylean was kindly provided by Eli Lilly and Company (Taiwan), Inc. Horseradish peroxidase (HRP) was from Roche (Switzerland). TMB plus ready-to-use substrate was obtained from Kem-En-Tec (Denmark). Methanol and acetonitrile were of liquid chromatographic grade. All other chemicals were of analytical-reagent grade and were used as obtained. Deionized water was purified on a Milli-Q system (Millipore, MA, USA).

**Buffers.** The buffers used were the follows: (A) coating buffer, 0.05 M carbonate/bicarbonate buffer solution, pH 9.6; (B) washing buffer, phosphate buffered with Tween 20, pH 7.4 were prepared with 0.01 M phosphate buffer, 0.0027 M KCl, 0.14 M NaCl, and 0.05% Tween. (C) dilution buffer: the same as buffer B. (D) PBS buffer: 0.01 M phosphate buffered saline (NaCl, 0.14 M; KCl, 0.0027 M), pH 7.4.

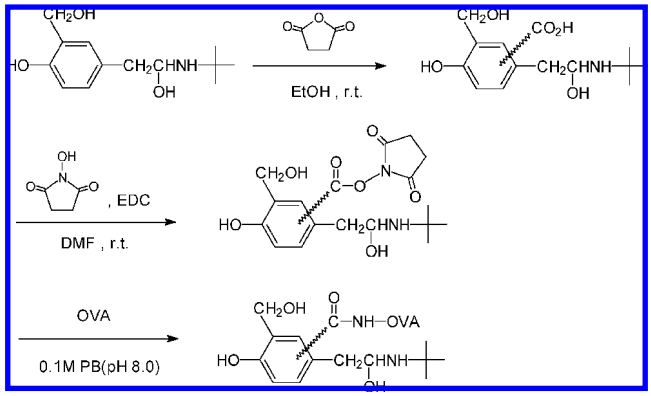
**Materials.** Ninty-six-well plates were obtained from Costar (Cambridge, MA, USA). Bond-Elut Certify columns were perchased from Varian Diagnostics (Harbor City, CA, USA). GC capillary column HP-1 was from Agilent (Santa Clara, CA, USA).

**Instrumentation.** A High Speed Refrigerated Centrifuge and a Tabletop Centrifuge (Kubota 6900 and 5400, Tokyo, Japan) were used. The antibody was dispensed in microtiter plates using a $\mu$Fill microplate dispenser (Bio-Tek, Winooski VT, USA). The microtiter plates were washed with the washing solution to remove unbounded antibodies using a 96PW microplate washer (Tecan, SLT., Salzburg, Austria). The absorbances of each well were measured with the EMax microplate reader (Molecular. Devices, Sunnyvale, CA). The serum extraction and cleanup for GC-Mass confirmation was performed using solid phase extraction vacuum manifold 12 Port (Supelco, USA) and TurboVap LV Concentration workstations (Caliper, Hopkinton, MA, USA). The Gas chromatograph−mass spectrometer used for analyzing the $\beta$-agonists was a 6890 GC/5973 MSD (Agilent, USA).

**Immunogen Preparation.** *a. Preparation of Salbutamol Succinate.* Salbutamol free base (2.39 g, 10 mmol) was dissolved in dry ethanol (20 mL) using a magnetic stirrer. While stirring, 1.2 g (12 mmol) of succinic anhydride was added. A cloudy white suspension appeared, and the formation of the salbutamol succinate was monitored by thin layer chromatography ($R_f$ salbutamol = 0.51; $R_f$ succinyl derivative = 0.21). After 30 min at room temperature, the reaction appeared complete, the stirring was stopped, and the suspension was centrifuged at 3,000*g* for 15 min. The solid phase was washed three times with diethyl ether and dried in vacuo at 50 °C for 5 h.

*b. Preparation of Immunogen by Coupling of Salbutamol Succinate to OVA.* After dissolution of 1.7 mg of salbutamol succinate (5 $\mu$mol), 0.7 mg of *N*-hydroxysuccinimide (6 $\mu$mol) and 1.4 mg of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (7.5 $\mu$mol) in 1 mL dry DMF were added. The mixture was stirred for 1 h at room temperature. OVA solution (16 mg in 4 mL of 0.1 N phosphate buffer, pH 8.0) was added dropwise to the above hapten mixture. The resultant solution was stirred for 1 h at room temperature, and the conjugate was

**Scheme 1.** Synthesis of Salbutamol Succinate and Coupling of Salbutamol Succinate to OVA




then dialyzed against 3 L of 0.01 M phosphate buffer solution containing 0.14 M NaCl for 1 day with 2 changes of buffer. The dialyzed solution of immunogen was frozen at −20 °C until use (**Scheme 1**).

*c. Antibody Preparation.* Five rabbits (New Zealand breed) were immunized by sc injection with salbutamol succinate conjugated to OVA. Primary immunizations were composed of 500 μL of PBS containing 1 mg of immunogen emulsified in 500 μL of Freund's complete adjuvant. Subsequent immunizations, at 2−5 week intervals, were of the same volume, with complete adjuvant replaced by incomplete adjuvant. Rabbits were bled after each injection. The salbutamol-specific antibodies were purified from high titer rabbit serum by affinity chromatography on a Protein A-Sepharose column. The antibodies were concentrated using Centriprep (Amicon Ultra, 50,000 MWCO; Millipore). Antibody concentration was measured with Bio-Rad protein assay.

**Homemade ELISA Kit.** A competitive enzyme immunoassay method for β-agonists has been developed using an antiserum raised in rabbits (New Zealand breed) by immunization against salbutamol derivative coupled to ovalbumin. The same derivative of the hapten was used, prepared by coupling to horseradish peroxidase to synthesize the enzymatic tracer. The 96-well microtiter plate was precoated with 0.9 μg/well antisalbutamol polyclone antibody overnight at 4 °C in coating buffer. The wells were blocked with 3% bovine serum albumin (BSA) in PBS for 2 h at room temperature the second day after washing them three times with washing buffer. The microtiter plates were dried at 20 °C, 25% RH for 4 h.

The quantitative β-agonist ELISA kit contains the following components: a 96-well microtiter plate (spilt into 12 strips of 8 wells each), precoated with antisalbutamol antibody, salbutamol−horseradish peroxidase enzyme conjugate, six salbutamol standard solutions (0, 0.05, 0.1, 0.25, 0.5, and 1 ng/mL), chromogen [3,3′,5,5′-tetramethylbenzidine (TMB)], dilution buffer, washing buffer, and stop solution.

**Evaluation of the GC-MS Method.** In order to evaluate LOD, linearity, and to calibrate the methods, standards were prepared by spiking pooled swine drug-free serum with salbutamol at concentrations of 1, 5, 10, 25, 50, and 100 ng/mL. Standards were prepared and analyzed on 5 separate days. Calibration curves were obtained from the analyte to internal standard peak area using linear regression. To determine intraday and interday precision as well as accuracy of the method, spiked serum samples at a concentration of 10 ng/mL were prepared and analyzed 6 times.

**Sampling.** The preliminary survey of the local market to detect the presence of salbutamol residues was conducted, and samples were collected from various sources by Technical Service Center, National Animal Industry Foundation (NAIF). The samples included 1,358 swine serums that were collected from the local meat markets (24 Fongshan district, Kaohsiung County, 92 Taoyuan county, 26 Pingtung county, 23 Hsinchu county, 15 Miaoli county, 25 Da-an District, Taichung county, 18 Nantou county, 30 Yunlin county, 16 Taipei county, and 13 Yilan county) and 1,076 from NAIF in Taiwan. The samples were processed to detect any salbutamol residues.

**Sample Preparation and the ELISA Procedure for Screening.** *1. Analysis of β-Agonist by Homemade ELISA.* Serum samples were diluted 1 + 4 with dilution buffer, centrifuged for 5 min at 3,000 rpm, and directly tested in ELISA, and 100 μL of standard (0, 0.05, 0.1, 0.25, 0.5 and 1 ng/mL salbutamol) or sample with 50 μL of salbutamol−enzyme conjugate was added to each well. Maximum binding was assessed by adding no inhibitor (zero standard) to the relevant wells. The plate was then incubated for 60 min at room temperature (19−25 °C) in the dark. After washing three times, the wells were emptied completely by inverting them onto absorbent paper; 100 μL each of chromagen (TMB) was added. The contents were mixed thoroughly and left to incubate for 20 min at room temperature in the dark. Afterward, 100 μL of stop solution (0.5 N HCl) was added to each well to stop the reaction. The absorbance was determined using a test wavelength of 450 nm against a reference wavelength of 650 nm. The contents of the wells were stable for 30 min. The system demonstrated a matrix effect similar to that of other enzyme immunoassays, the dilution of serum decreased and eliminated the matrix effect.

*2. Analysis of β-Agonist by Commercially Available ELISA.* A competitive enzyme immunoassay obtained from Randox, UK, was used. Serum samples were diluted 1 + 4 with diluent/wash buffer, centrifuged for 5 min at 3,000 rpm, and directly tested in the ELISA, and 50 μL of standard (0, 0.09, 0.19, 0.42, 0.9, and 4.7 ng/mL clenbuterol) or sample with 100 μL of conjugate was added to each well. The microtiter plate was gently tapped from side to side and incubated for 1 h at room temperature (19−25 °C). The plate was inverted and the liquid tapped out. After washing six times, it was tapped out onto tissue paper ensuring that all diluents/wash buffer were removed. One hundred twenty-five microliters of the one shot substrate solution was pipetted into each well using a multichannel pipet. The microtiter plate was gently tapped from side to side and incubated for 20 min at room temperature in the dark. Afterward, 100 μL of stop solution (0.2 M $H_2SO_4$) was added to each well to stop the reaction. The absorbance was determined using a test wavelength of 450 nm, within 10 min.

*3. Calculation.* Results were calculated by interpolation from the calibration curve where the bound enzyme activity was expressed as the logit of the ratio (in present). Each concentration of salbutamol ($B$) and the bound activity in the absence of unlabeled salbutamol ($B_0$) was plotted versus the log of salbutamol concentration.

**Sample Preparation for GC-MS.** *1. Serum (Plasma) Extraction.* Serum or plasma samples (2 mL), spiked as stated above with salbutamol-*tert*-butyl-$d_9$, were placed in 30-mL screw-capped tubes with 0.4 mL of 0.5 N HCl. After brief mixing, pH was adjusted to 9.1 with 1 N NaOH. Then the tubes were shaken or vertexed for 10 s. Then they were centrifuged for 10 min at 40,000*g*, and the samples were stored until they were loaded onto the Bond-Elut column.

*2. Extraction with Bond-Elut Certified Columns.* A 3-mL (500 mg) Bond-Elut certified disposable extraction column was activated with 10 mL of methanol followed by 10 mL of deionized water, all at 3 mL/min. Care was taken to prevent air from reaching the stationary phase in this step. The serum (plasma) extraction was now loaded onto the column and collected to waste. The column was then washed with 10 mL of deionized water at 3 mL/min and dried with vacuum state for 20 min. Subsequently, the column was washed with 10 mL of acetonitrile at 3 mL/min and dried with vacuum state for 20 min. Elution was then carried out with 2 mL methanol at 3 mL/min and was decanted into a 10-mL Quickfit glass tube. The extract was evaporated under a gentle steam of nitrogen at 50 °C.

*3. Derivatization.* The dry residue was dissolved as described above in 50 μL of MSTFA at 70 °C for 60 min. A 1-μL aliquot of each MSTFA-derivatized sample was injected into the gas chromatograph−mass spectrometer via splitless injection.

*4. GC-MS Analysis.* GC-MS of the β-agonists was performed according to Chinese National Standards (CNS) General No.14619, Catalog No.N4187, Determination of β-Agonists Salbutamol, Terbutaline and Clenbuterol in Feeds, Bureau of Standards Metrology and Inspection, M.O.E.A., ROC. The column and MS conditions are described in **Table 3**. For the purpose of confirmation of the identity, additional (fragments) ions must be monitored. TMS derivatives of the appropriate β-agonist (salbutamol-3TMS, terbutaline-3TMS, and clenbuterol-TMS) fragments, the masses of the ions, and the ion ratios used for identification criteria are summarized in **Table 4**.

**Table 3.** Apparatus and GC-MS Conditions Used for Salbutamol Analysis

| | |
|---|---|
| GC-MS | Agilent 6890 series |
| column | HP-1 fused-silica capillary column<br>length 30 m, 0.25 mm I.D., film thickness 0.25 $\mu$m |
| injection pressure | 2.9 psi |
| column gas flow rate | He, 0.56 mL/min |
| injector temperature | 280 °C |
| detector temperature | 310 °C |
| oven program | initial 70 °C (remain 1 min)<br>program 30 °C/min<br>middle 230 °C (remin 5 min)<br>program 30 °C.min<br>final 300 °C (remain 6 min) |
| injection mode | splitless |
| injection volume | 1 $\mu$L |
| detection mode | selective ion monitoring (SIM) |
| electron energy | 70 eV |

**Table 4.** Ions and Ratios for GC-MS Analyses of $\beta$-Agonists-TMS Derivatives[a]

| compound | $N$[b] | $M_0$[c] | $M_d$[d] | ions | | | |
|---|---|---|---|---|---|---|---|
| salbutamol | 3 | 239 | 455 | 86 (100) | 350 (2.3) | 369 (94.4) | 371 (16.2) |
| terbutaline | 3 | 225 | 442 | 86 (100) | 336 (2.8) | 356 (67.0) | 358 (10.9) |
| clenbuterol | 1 | 277 | 348 | 86 (100) | 243 (8.1) | 262 (16.5) | 264 (10.9) |

[a] The number in parentheses indicates the relative peak intensity. [b] $N$ = number of TMS groups. [c] $M_0$ = molecular mass. [d] $M_d$ = molecular mass after derivatization.

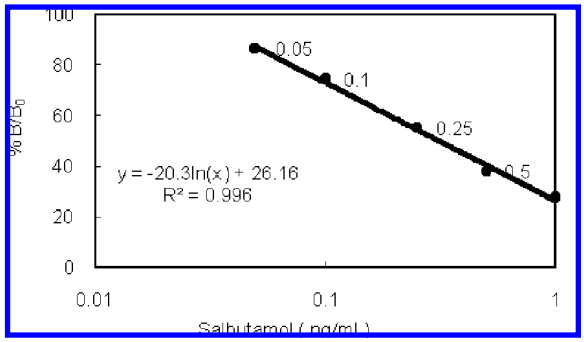



**Figure 1.** Calibration curve for salbutamol ELISA. $B$ = absorbance of each standard or sample, $B_0$ = absorbance of standard = control.

## RESULTS AND DISCUSSION

**Analytical Performance of Homemade ELISA Kit.** A typical standard curve obtained using the homemade ELISA is presented in **Figure 1** The result of the salbutamol-ELISA produced linear ranges from 0.05 to 1 ng/mL.

In order to assess the assay as a serum test, we examined the specificity, sensitivity, recovery, and precision of the assay with swine serum samples.

Specificity of the assay was estimated by measuring cross-reactivity with 5 commonly used $\beta$-agonists. Besides good binding for salbutamol (100%), there was a group of substances that showed affinity to the antibody (cross-reactivities as calculated at 50% relative binding). Specificity experiments indicated 100% cross-reactivity with salbutamol, 70% with clenbuterol, 90% with terbutaline, 29% with isoproterenol, and 0.05% with Paylean (**Table 5**). The main cross-reacting structurally related compounds are clenbuterol and terbutaline, while other studied compounds such as isoproterenol and Paylean did not significantly cross-react.

The sensitivity of the assay was evaluated by examining swine serum samples, and the limit of detection of this assay was estimated to be 0.052 ng/mL (mean determined concentration of 20 blank serum samples collected from untreated swine + 3 times standard deviation). The limit of determination (mean of 20 blank swine serum samples + 10 times standard deviation) was 0.26 ng/mL.

**Table 5.** Specificity of Salbutamol Antibody with Selected $\beta$-Agonists Using Homemade ELISA

| compound | % cross-reactivity |
|---|---|
| salbutamol | 100 |
| clenbuterol | 70 |
| terbutaline | 90 |
| isoproterenol | 29 |
| Paylean | 0.05 |

**Table 6.** Recovery of Salbutamol Added to Swine Serum Samples with Homemade ELISA ($n$ = 3)

| spiking concentration (ng/mL) | recovery (%) |
|---|---|
| 0.50 | 90−120 |
| 1.00 | 85−114 |
| 2.00 | 91−115 |

**Table 7.** Intra- and Interbatch Variations in Swine Serum Salbutamol Measurements with Homemade ELISA

| spiking concentration (ng/mL) | mean[a] ± SD (ng/mL) | C.V.[b] (%) |
|---|---|---|
| | Intra-Assay (10 Replicates) | |
| 0.50 | 0.50 ± 0.03 | 5.3 |
| 1.00 | 1.02 ± 0.05 | 5.0 |
| 2.00 | 2.06 ± 0.12 | 6.0 |
| | Inter-Assay (10 Replicates) | |
| 0.50 | 0.51 ± 0.04 | 8.7 |
| 1.00 | 1.08 ± 0.09 | 8.5 |
| 2.00 | 2.09 ± 0.16 | 7.5 |

[a] Salbutamol concentration found. [b] Coefficient of variation.

Recovery was investigated by adding increasing amounts of salbutamol (0.5, 1, and 2 ng/mL) to swine serum samples. The results indicated a recovery ranging from 85 to 120% (**Table 6**). Mean recovery thus indicated a reasonable parallelism and accuracy of the assay when applied to real samples.

Precision of the assay was assessed by replicate measurements of three known swine serum samples with a final salbutamol concentration of 0.5 ng/mL, 1 ng/mL, and 2 ng/mL. The obtained mean values ± SD and C.V. (%) by replicate analyses ($n$ = 10) in the same run (intra) and in separate runs (inter assay) are reported in **Table 7**. The CV % values were below 10%, demonstrating an acceptable level of precision. The use of an automated dispensing system helps improve the quality of the assay.

**Analytical Performance of GC-MS.** For salbutamol, the range of linearity has been verified on spiked swine serums (salbutamol concentration: 1−100 ng/mL). The response is linear with a correlation factor $R^2$ = 0.9951. The value of the limit of detection (LOD) was 0.1 ng/mL, and the limit of quantitation (LOQ) was 0.35 ng/mL. Precision and accuracy of the method were calculated at a concentration level of 10 ng/mL. Intraday and interday precision coefficients of variation (CV) were low, and results were accurate (**Table 8**). Terbutaline and clenbuterol can be detected to 0.1 ng/mL and 0.5 ng/mL, respectively.

**Application of Homemade and Commercially Available ELISA Kits in Field Trial Measurements.** Field trials with 1,358 swine serum samples from local meat markets were analyzed. The qualitative immunoassay reports each sample as either positive or negative, on the basis of predetermined cutoff concentrations.

**Table 8.** Precision and Accuracy of the GC-MS Method Determined Using Swine Serum Samples Spiked at 10 ng/mL of Salbutamol

| spiking concentration (ng/mL) | mean[a] ± SD (ng/mL) | C.V.[b] (%) |
|---|---|---|
| | Intraday Assay (10 Replicates) | |
| 10 | 9.6 ± 0.32 | 3.39 |
| | Interday Assay (10 Replicates) | |
| 10 | 10.1 ± 0.52 | 5.20 |

[a] Salbutamol concentration found. [b] Coefficient of variation.

**Table 9.** Comparison of Homemade and Commercially Available ELISA with 1,358 Swine Serum Samples from Local Meat Markets

| immunoassay screening cutoff concentration (1 ng/mL) | confirmation cutoff concentration (1 ng/mL) | no. of samples | |
|---|---|---|---|
| | | homemade | commercially available |
| +[a] | +[a] | 30 | 35 |
| −[b] | +[a] | 7 | 2 |
| +[a] | −[b] | 19 | 177 |
| −[b] | −[b] | 1302 | 1144 |
| sensitivity[c] | | 81.1% | 89.2% |
| specificity[d] | | 98.6% | 86.7% |

[a] Positive test result. [b] Negative test result. [c] % Sensitivity = (true positives − false negatives)/(true positives) × 100%. [d] % Specificity = (true negatives − false positives)/(true negatives) × 100%.

In the ideal diagnosis, results would be positive if the animal took the drug (true positive) and negative if the drug was not taken (true negative). However, false-positive or false-negative results can occur; therefore, it is imperative to interpret the results carefully. Testing technology is constantly evolving and varies by manufacturer; flase-positive or false-negative results today may not be relevant in the future. In context, the sensitivity of a test is the ability to detect a class of drug, while the specificity is the ability to identify a particular drug. A high specific test gives few false-positive results and identifies individual drugs and/or their metabolites. High sensitivity is due to the test's ability to detect the drug and/or its metabolite(s) and to reach the cutoff concentration for a positive report. The method was found to be suitable for use in Taiwan's meat market, and a cutoff limit of 1 ng salbutamol/mL swine serum was set by the authorities, Council of Agriculture, Executive Yuan, ROC, and administered by the Bureau of Animal and Plant Health Inspection and Quarantine (BAPHIQ). According to the recommended cutoff concentration of salbutamol, results were determined to be positive or negative. The sensitivity is the proportion of true positives, and the specificity is the proportion of true negatives (*19*). The homemade salbutamol ELISA had a sensitivity of 81.1% and a specificity of 98.6% versus GC-MS at a cutoff of 1 ng/mL. The commercially available β-agonist ELISA had a sensitivity of 89.2% and a specificity of 86.7% versus GC-MS (1 ng/mL salbutamol cutoff) (**Table 9**).

False-positive results can be reported because of cross-reactivity with other compounds found in food producing animals. GC-MS is not influenced by cross-reacting compounds. All positive results with the swine serum were reviewed to explore possible explanations. All unexpected results should be verified with the laboratory to ensure their accuracy. A false-negative result is technically defined as a negative finding in a sample known to contain the drug of interest. This may occur through laboratory or clerical error, or, less likely, through damage with the test sample. In our case, false-negative results may lead to concern about the predetermined scale cutoff points. The source data review we've done indicated that the false-negatives had values just over 1 ng/mL and that the kit assay calculations reached the borderline and the deviations below the cutoff of 5−10%. However, the false-negative rate and the false-positive rate are dependent: to decrease one is to increase the other. Decreasing the initial screening level would eliminate false-negative screens, which would increase the proportion of false-positive and significantly increase the laboratory expense by the need for costly, confirmations by GC-MS. The cutoff thresholds of the tradeoffs that minimize the joint occurrence of the two errors should be redefined in field applications in the future.

Objectives of the study were to develop a new assay and to compare with a commercially available kit in field test screen. The Randox β-agonist ELISA test kit was evaluated for the screening of serum for salbutamol residues in field samples, and the tests were described as sufficiently sensitive and reproducible. In response to our concern, the analysis of salbutamol in the swine serum is the more practical basis for large scale surveillance programs in Taiwan. A homemade salbutamol ELISA test kit showed a specificity of 98.6% and gave a low proportion of false-positive rate results. On the basis of the fact that the false-positive is equal to 1 minus the specificity the test, the number of false-positives reported by the Randox assay was nearly 10 times higher than the assay we developed. In this article, we present findings on the developed assay with regard to excellent specificity of the salbutamol measurements. We could screen to the number of samples submitted to time-consumimg sample cleanup, and chromatography analysis was considerably reduced.

In conclusion, the described enzyme-linked immunosorbent assay kit for the quantitative determination of β-agonist salbutamol in swine serum showed appreciable accuracy and precision. The serum sample preparation procedures are simple and rapid. From the practical point of view, the kit could be advantageously utilized to the screening of large groups of serum samples, and the kit employed has good reliability even in routine application for the control of the illegal use of the drug. This salbutamol ELISA kit is now commercially available (Taiwan Advance Bio-Pharm Inc., Taiwan).

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