

Enzyme Immunoassay of Sulfamethoxazole in Chicken Tissues: Interlaboratory Study

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(Received 6 June 1994/Accepted 11 August 1994)

ABSTRACT. Three laboratories participated in the interlaboratory study of an enzyme immunoassay (EIA) method for determination of sulfamethoxazole (SMX) using samples of the same muscle, liver and plasma from chickens administered with SMX. Interlaboratory variation in the determined values were similar to those of other studies determined by gas chromatography and liquid chromatography. It was suggested that the interlaboratory difference in determined SMX residues from chicken tissues did not become problems of using EIA method, in spite of the differences of equipments and operators.—**KEY WORDS:** enzyme immunoassay, interlaboratory study, sulfamethoxazole residue.

J. Vet. Med. Sci. 56(6): 1207–1208, 1994

Enzyme immunoassay (EIA) is a new testing method which has been used recently as a rapid and simple method for determining residual veterinary drugs in livestock products [4–9]. Measuring kits have already been available. Sulfamethoxazole (SMX) is one of sulfonamide drugs which is widely used as a prophylactic or therapeutic drug for chicken coccidiosis.

We have reported the applicability of EIA for determination of drug residues in animal tissues using SMX as a test drug [3]. It was suggested that EIA was applicable as a screening method for detecting SMX residues in chicken muscle.

In application of EIA for residue analysis, the precision and reproducibility of measurements of quantitative analysis become problems of extraordinary importance in case of judging presence or absence of residual drugs. Especially, fears of the interlaboratory differences of judgment due to different apparatus and operators are entertained. However, any interlaboratory studies for EIA analysis have not yet been reported.

This trial was conducted in three laboratories. They received same frozen samples and the same EIA kit with same direction for analytical procedure. Operators were not always familiar with this EIA system.

SMX-EIA test kit (Mitsubishi Yuka BCL, Tokyo, Japan) was used as a model of EIA system. It consists of anti-SMX antibody coated microtiter plate (96 well), peroxidase conjugated SMX, standard solutions of SMX, wash solution, extracting solution, citrate buffer solution, substrate and stop solution for enzyme reaction. Wash solution was diluted 10-fold with de-ionized water. Extracting solution was diluted with diluted wash solution and substrate was dissolved in citrate buffer solution.

Instruments for sample preparation and detection in each laboratory were as follows.

Laboratory A: A model of UR-150 P ultrasonic homogenizer (Tomy Seiko Co., Ltd., Tokyo, Japan), a model of sun washer 96 microtiter plate washer (Sanko Junyaku Co., Ltd., Tokyo, Japan), a model of MTP-32 microtiter plate reader (Corona Electric Co., Ltd., Tokyo, Japan).

Laboratory B: A model of Bio-mixer high-speed homogenizer (Niti-On Medical & Physical Instruments

MFG., Co., Ltd., Chiba, Japan), a model of MTP-100 microtiter plate reader (Corona).

Laboratory C: A model of UR-200 P ultrasonic homogenizer (Tomy), a model of ER-8000 microtiter plate reader (Sanko Junyaku).

Tissue samples were muscles, livers and plasma from chickens (9 weeks old White Leghorn, Nisseiken Co., Ltd., Tokyo, Japan) administered with 10–100 mg/kg sodium SMX (Shinomin sodium, Shionogi & Co., Ltd., Osaka, Japan) in water orally by catheter. At 24 and 48 hr after administration of SMX, blood samples were collected in heparinized tubes, and muscles and livers were removed from chickens sacrificed by bleeding. Blood samples were centrifuged for 10 min at 3,000 rpm. Each tissue and plasma sample was divided into three portion, and stored at –80°C. Control samples were prepared by the same procedure.

The frozen samples were subsequently packed with ice and shipped to the laboratories with instructions that they be kept frozen until analysis.

In each laboratory, 11 muscle samples, 11 liver samples and 5 plasma samples from chickens administered with SMX, and muscle, liver and plasma samples from control chickens were analyzed by the EIA kit.

Amounts of 0.5–1.0 g (ml) of each sample was homogenized with 7-fold extracting solution and centrifuged at 3,000 rpm for 15 min. The clear supernatant was used as a test solution. Each test solution and standard solutions of SMX was mixed with peroxidase conjugated SMX solution. After that, 100 μ l of each mixed solution was put into the well of the microtiter plate coated with the anti-SMX antibody, the plate was incubated at room temperature for 1 hr. After incubation all wells were emptied, washed with the wash solution, and placed 100 μ l of the substrate solution. The plate was incubated at room temperature for 30–45 min until color development was enough. Then, 100 μ l of the stop solution was pipetted into each well to stop the coloring reaction, and optical absorption value of each well at 415 nm was measured. Each sample was analyzed in duplicate, and the mean value was used to calculate SMX concentration. The range of quantitative analysis of SMX concentration with this kit was 10–2,000 ng/g (ml).

Table 1. Sulfamethoxazole (SMX) concentrations (ng/g (ml)) in tissues from chickens administrated SMX

Sample	SMX concentration ^{a)}			SMX concentration ^{b)}	
	Lab. A ^{c)}	Lab. B	Lab. C	Lab. A	Lab. B
Muscle					
1	>2000	1900	1860	>2000	>2000
2	680	520	550	950	950
3	400	520	450	700	950
4	270	240	210	270	390
5	52	30	70	40	50
6	19	<10	10	<10	<10
7	<10	<10	10	<10	<10
8	<10	<10	20	<10	<10
9	<10	<10	10	<10	<10
10	<10	<10	<10	<10	<10
11	<10	<10	<10	<10	<10
Liver					
1	>2000	>2000	>2000	>2000	>2000
2	>2000	>2000	1720	>2000	>2000
3	>2000	>2000	1770	>2000	>2000
4	1700	1750	1150	1600	>2000
5	800	1100	750	1100	1350
6	860	1350	1090	1150	1600
7	230	280	310	300	290
8	330	450	440	420	440
9	60	100	120	130	60
10	270	170	330	330	130
11	77	40	80	70	10
Plasma					
1	17	<10	10	10	<10
2	10	<10	10	<10	<10
3	<10	10	10	<10	20
4	<10	<10	<10	<10	<10
5	<10	<10	<10	<10	<10

a) Calculated by the calibration curve made by SMX standard solution.

b) Calculated by the calibration curve made by the addition of SMX to control tissues.

c) Collaborators.

The determined SMX concentrations of tissue samples from chickens administered with SMX in each laboratory are shown in Table 1. SMX concentrations determined by the calibration curve made by SMX standard solutions were studied in three laboratories. Those determined by the calibration curve made by adding SMX to control tissues were studied in only two laboratories because of a mistake about procedure adding SMX to control tissues.

The determined SMX concentrations of same samples obtained in each laboratory were a little different. However, interlaboratory coefficients of variation of 12 samples, of which concentrations were determined within

the quantitative range of 10–2,000 ng/g (ml) by the calibration curve made by SMX standard solutions in three laboratories, were ranged from 2.2 % to 39.2% (mean $\pm$ SD = 21.2 $\pm$ 11.0%). This result was not different from those reported in other residual interlaboratory studies with positive residue tissue samples. Those of clopidol detected with gas chromatography (GC) were ranged from 14.8% (muscle) to 21.9% (liver) [2]. Those of chloramphenicol detected with high-performance liquid chromatography (HPLC) from muscles were ranged from 15.7% to 48.2% (overall mean = 17.9%) [1]. Those of sulfamethazine detected with HPLC from raw milk were 21.3%, 22.9% and 31.9% [10]. It was suggested that the interlaboratory difference of concentrations determined by the SMX-EIA kit was almost similar to those determined by GC and HPLC which were common chemical analytical methods for drug residues from animal tissues.

Further study concerning the comparison of determined SMX concentrations by EIA with those by chemical analysis is still needed to ascertain that these concentrations are correct.

ACKNOWLEDGEMENTS. The authors wish to thank Dr. Y. Kido (Research Institute for Animal Science in Biochemistry and Toxicology), Dr. M. Fujita (Mitsubishi Yuka BCL) and their staffs for their participation in this study.

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