Introduction and Background

A. Overview

The antibiotic Sulfamethazine, 4-amino-N-(4,6-dimethyl-2-pyrimidinyl) benzene sulfonamide, is commonly incorporated into swine feed as promotants of growth and for control of certain diseases in animals. Consumption of meat from animals containing the sulfonamide antibiotics residues may result in development of hypersensitivity to these drugs. In addition, it may allowed the preferentially selection of bacterial mutants that are resistant to these antibiotics. Tissue residues in animals are controlled by withdrawing the antibiotics from feed weeks before their slaughter when concentrations of the sulfonamides are presumed to reach below their safety level. However, in order to ensure a residue-safe meat reaching the public, it is recommended to check the animals before they are slaughtered. The sulfamethazine EIA kit is a rapid and simple detection system for sulfamethazine in swine plasma or serum. The sensitivity of this assay is excellent, and is well suited for the determination of sulfamethazine in blood, urine and other sample extracts.

B. Test Principle

The enzyme immunoassay for sulfamethazine is based on the competition between the sulfamethazine to be assayed and the sulfamethazine-alkaline phosphatase conjugate, for binding to rabbit antibody directed against sulfamethazine, coated onto microwells. The sample containing the sulfamethazine, and the sulfamethazine-alkaline phosphatase conjugate, when added to the microtiter wells, compete for binding to a limiting number of antibody sites. After incubation, each well is rinsed in order to remove non-bound components. The bound enzymatic activity is then measured by the addition of a chromogenic substrate. The intensity of the color developed is inversely proportional to the concentration of sulfamethazine in the sample. The concentration is calculated on the basis of a standard curve.

Material and Method

A. Reagents

*Reagents are for **in vitro research use** only. All reagents of the kit are stable, if stored at 2-8°C, until the expiration date stated on the kit.*

1. 96-wells microtiter plate **(#S**). Twelve strips of 8 detachable wells, coated with rabbit Anti-Sulfamethazine antibody.
2. One vial containing 0.25 ml of 1 µg/ml sulfamethazine.
3. One bottle **(#3)** containing 9.5 ml of Sulfamethazine-Alkaline Phosphatase conjugate (SMZ-ALP).
4. One bottle **(#5)** containing 9.5 ml of p-Nitrophenyl Phosphate (pNPP). Ready to use.
5. One bottle **(#6)** containing 15 ml of Wash Buffer (10xPBS-Tween). Dilute 10 fold with distilled or deionized water to 150 ml prior to use.
6. One bottle **(#7)** containing 5.5 ml of Stop Solution, 3N NaOH.

B. Optional Equipment and Material Required

1. Pipetters capable of delivering 50 µl and 100 µl.
2. Microtiter plate reader (wavelength 405 nm).
3. Plate washer or squeezable wash bottle.
4. Timer.
5. Absorbent paper towels.

C. Protocol

Let the components of the kit equilibrate to room temperature before use.

1. Carefully add **25 µl** of standard or sample to the bottom of each well. Slightly tap the side of the strip holder to evenly distribute the sample.
2. Avoid touching the well with pipette tip and add **100 µl** of SMZ-ALP conjugate **(#3)** to each well. Slightly tap the side of the strip holder to properly mix the sample and enzyme conjugate.
3. Incubate at room temperature for **40 minutes**.
4. After incubation, dispose the solution in the wells by inverting and shaking. Wash microtiter wells **3 times** with wash buffer **(#6)** to remove the non-bound conjugate. Washing may be done manually as follows: use squeeze bottle to fill wells gently with wash buffer, dumping the wells between each wash by inverting and shaking. After the third wash, tamp holder with washed strips onto a piece of absorbent paper.
5. Add **100 µl** of pNPP substrate **(#5)** to each well and incubate at room temperature for **20 min**. To avoid contamination, place the needed amount of substrate into a test tube and dispense only from the tube itself.
6. Add **50 µl** of Stop Solution **(#7)** to each well and tap the strip holder for proper mixing.
7. Read absorbance at **405 nm** using an ELISA reader.

Preparation of Standard Curve

1. Calculation
   (a) Average the absorbance (OD_s) for each standard concentration of sulfamethazine including 0 ng/ml (OD_0).
   (b) % of Inhibition = $100 - (OD_s / OD_0) \times 100$
2. Plot values of % of Inhibition, step 1 (b), against their corresponding concentrations on Log_{10} paper.
3. Calculate sulfamethazine concentration of sample by interpolation and multiply by dilution factor to obtain the actual quantity of sulfamethazine.

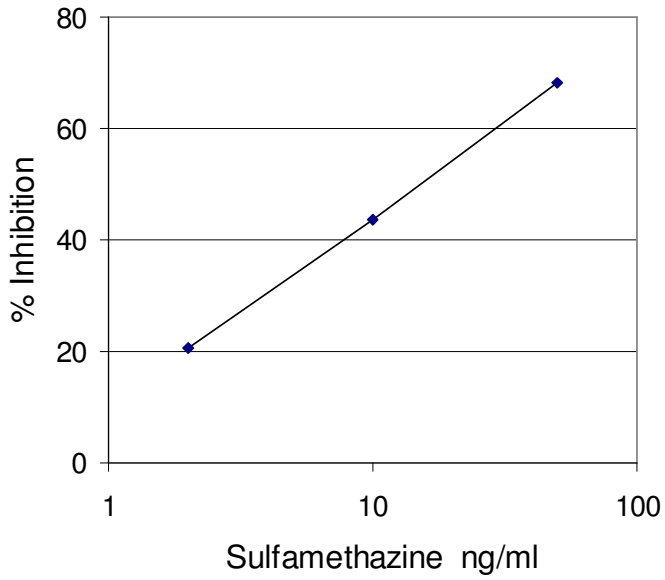
General Remarks

1. Do not mix reagents from different lots.
2. If concentrations of sulfamethazine in the samples are high, dilute sample such that points fall in the middle range of the standard curve.
3. Do not return unused reagents back into their original bottles.
4. Samples tested should have a pH of 7.0 (± 1.0). Excessive alkaline or acidic conditions may affect the test results.
5. The stop solution contains NaOH. Do not allow to contact skin or eyes. If exposed, flush with water.
6. Dispose of all materials, containers and devices in the appropriate receptacle after use.

Simplified Assay Procedure

1. Add sample and standard (25 µl).
2. Add Enzyme conjugate (100 µl). 40 min at RT.
3. Wash 3x.
4. Add pNPP (100 µl), wait for 20 min at RT.
5. Add stop solution (50 µl) and read at 405 nm.

Sulfamethazine Inhibition curve



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