VALIDATION OF DEVELOPED ANTIBODY USING SURFACE PLASM ON RESONANCE FOR DETERMINATION OF NITROFURAN RESIDUES IN POULTRY

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Polyclonal antibodies (pAb) were produced to detect semicarbazide (SEM), a metabolite as a marker residue of nitrofurazone in animal food production. A carboxyphenyl derivative of SEM was synthesized following derivatisation with 4-carboxybenzaldehyde (CBA). Modified SEM was purified and conjugated to bovine serum albumin (BSA) or ovalbumin (OVA) as immunogen and coating antigen, respectively. The titer determination of the pAb to SEM was 0.01 mgmL–1. pAb is suitable to develop an immunoassay for SEM with sufficient sensitivity for monitoring nitrofurazone residues. An alternative determination of developed antibody was used for comparison purposes. It will also perform as the validation tool to determine response from the developed antibody to the antigen similarly as the immunoassay method. It also can produce rapid and reliable results. For this reasons, biosensor specifically Surface Plasmon Resonance (SPR) are chosen to comply with this needs. Nitrofurazone is a broad-spectrum anti-bactericidal drug and belongs to the class of nitrofuran. It also possesses some anti-protozoal activity and is used both therapeutically and prophylactically in a number of food-producing animal species including pigs, sheep, goats, cattle, chickens and turkeys as a growth promoter (1). Because nitrofuran drugs have potentially harmful effects on human health, the European Union has prohibited their use in food-producing animals (2). Nitrofuran drugs are rapidly metabolized in vivo and do not persist as residues of the parent drugs in edible tissues (3, 4). The metabolites of these drugs bind to tissue proteins and persist for onsiderable periods in animal tissues after treatment and the protein-bound residues are stable in tissue matrices, even after long-term storage (5–7). In the case of the nitrofuran drugs furazolidone, furaltadone, nitrofurantoin and nitrofurazone, a proportion of the bound metabolites possess the intact side chain 3-amino-2-oxazolidone (AOZ), 5-morpholino-3-amino-2-oxazolidone (AMOZ), 1-aminohydantoin (AHD) and semicarbazide (SEM), respectively [8]. These residues may be released as potentially toxic entities in the acidic conditions of human stomach [9]. SEM is a metabolite of nitrofurazone and has been used as a marker residue for illegal use of this drug in animal food production (10, 11).This approach is based on polyclonal antibodies against semicarbazide.

Determination of polyclonal antibody against semicarbazide using synthesized hapten has been done using specific binding between an antigen and antibody is measured solid surface reaction Surface plasmon resonance (biosensor device) was performed using AutoLab SPR from Metrohm. This method was used to get the validity and specificity of the newly developed IgG.

Research has been done on the concentration on immobilization of SEM-KLH, where 1.0mg/ml of antigen were immobilized. Figure 1 shows sensitive response than ELISA in Figure 2. Concentration as low as 0.1 x 10^{-7} mg/ml of anti-semicarbazide can be detected using this method. This also proven that SPR is more sensitive than ELISA method where detection limit at only 0.01mg/ml of anti-

semicarbazide and can be an alternative method for detection of nitrofuran antibiotics. From this study, surface plasmon resonance (SPR) was proven to be an alternative method for the detection of nitrofurans antibiotic in poultry meat. Its sensitive response against anti-semicarbazide is as low as 0.1 x 10^{-7} mg/ml shows that it is more sensitive compared to ELISA method which its detection limit were only up to 0.01mg/ml (Figure 2) . Thus an urgent need of rapid, fast and cost effective detection methods to fulfil the needs for monitoring nitrofuran contamination

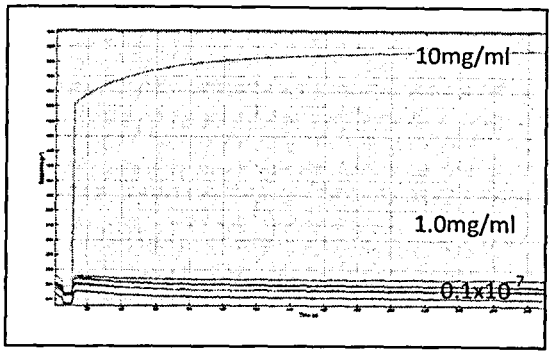

Figure 1: Response of the Biosensor to Various Concentration of Anti-Semicarbazide with immobilization of 1.0mg/ml SEM-KLH

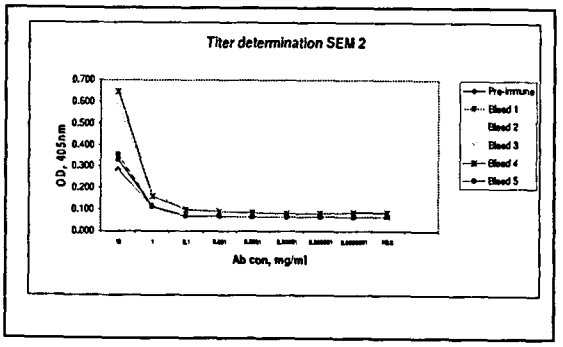

Figure 2: Link Immunosorbent Assay (ELISA) for SEM2

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