DEVELOPMENT OF ONE-STEP IMMUNOCHROMATOGRAPHIC STRIP FOR THE DETECTION OF TOTAL AFLATOXIN IN CORN SAMPLE BASED ON MONOCLONAL ANTIBODY CLONE 4G6

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ABSTRACT

Aflatoxins are cancer-causing chemicals produced primarily by *Aspergillus flavus* and *A. parasiticus*. Aflatoxin B1 (AFB1) is the most commonly found aflatoxin in improperly stored staple commodities such grain and feed. Its presence in the food supply, can be carried over to animal products such as meat, liver, kidney, pig blood and milk. A specific and sensitive detection method is required for preliminary screening of these samples. This research sought to develop a detection kit for total aflatoxin by immunochromatographic technique using monoclonal antibody (MAb) from the hybridoma cell line 4G6. The experiments were conducted at the Serology and Diagnostic Laboratory, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom province during 2014-2016. The MAb is composed of IgG_{2b} isotype and lambda light chain. Its specificity recognized four aflatoxins including AFB_1, AFB_2, AFG_1 and AFG_2 with cross reactivity at 100%, 89.2%, 82.6%, and 72.7%, respectively by direct competitive enzyme-linked immunosorbent assay (dcELISA). *In vitro* propagation of the hybridoma was carried out using an Integra CELLine Culture System and the antibody was purified by affinity column chromatography. The conjugate probe was prepared by comparing two sizes of colloidal gold particles at 20 and 40 nm in diameter for the conjugation with the MAb. The MAb conjugate with 40 nm colloidal gold was selected and sprayed onto the conjugate release pad (CRP). The target cut-off value for the developed immunochromatographic strip (ICS) was 20 ng/mL according to a regulation limit in Thailand. The study on the appropriate conditions for this strip showed that aflatoxin B_1 conjugated to bovine serum albumin (AFB_1-BSA) and goat anti-mouse immunoglobulin (GAM) should be immobilized at the test line and control line at the same concentrations of 0.25 mg/mL. The testing sample was extracted with 70% methanol and further diluted 1:4 with Tris buffer saline with 0.05% Tween-20 (TBST) before application on the sample application pad (SAP) and the reaction could be visualized within 15 min. The analysis of 5 naturally contaminated corn samples (n=7) indicated that 2 samples contained ≥ 20 µg/kg and 3 samples contained < 20 µg/kg. Five samples, analyzed by dcELISA, showed contamination levels at <4, 9.6, 19.9, 10.5 and 39.7 µg/kg which delivered a good correlation to the results from ICS analysis.

Key words: rapid test kit, mycotoxin, toxin analysis, lateral flow assay