Abstract

Avian Influenza Virus is becoming the important public health problem in Thailand since the first emergent on 2003-2004. Worldwide, the total of 385 human cases of Avian Influenza Virus with 243 deaths were reported from WHO, which the total of 25 cases and 17 deaths came from Thailand. Moreover, it also caused a high number of economic losses in Asia including Thailand. For this reason, the diagnostic kit for this H5N1 avian influenza virus was set to be the one of national policy for controlling this disease. For production of diagnostic kit of this avian influenza virus antigen, monoclonal antibody has been well known as diagnostic reagent. To produce the monoclonal antibody, both traditional hybridoma technology and phage display technology can be applied, however, the latter is considered to be a much more feasible and cheaper. So, the objective of this study is construction and selection of Fab antibody phage library specific to H5N1 avian influenza virus using phage display technique. ***Methods*** – The immunized chimeric Chicken/Human Fab library were constructed with library size at 1.0×10^6. Three white leg horn chickens were immunized with the dead H5N1. The chicken spleens were harvested and pooled together. Total RNA were then isolated from these chicken spleens using TRIZol reagent. Then, complementary DNA (cDNA) were reverse transcribed from the RNA. cDNA were further used as template for antibody gene amplification specific to chicken variable heavy chain (V_H) and light chain (V_λ). Moreover, human constant heavy chain (C_H1) and light chain (CK) were also amplified from pComb3XTT vector. After that, overlap extension PCR were performed to amplify the heavy chain Fd (VH- C_H1), Chimeric light chain (V_λ – C_K), and final Fab fragment. This Fab were then digested with restriction enzyme *Sfi*1 and ligated to phagemid vector pComb3XSS that is also previously cut with the same enzyme. The recombinant DNA was transformed in to ER2738 *E. coli* host cell, and rescue with wild type helper phage VCSM13. According to panning strategy with immobilized antigen, the hemagglutinin-specific antibodies were selected from a repertoire of this chimeric Fab library. ***Result and Discussion*** – Fab antibody library from H5N1 immunized chicken with a library size at 1.0×10^7 was successfully constructed and used for selection in panning. From panning, the specific phage clones were enriched in consecutive round tested by phage pool ELISA. Totally 86 clones were selected for single clone Fab ELISA. Among these clones, we found 3 clones that give positive result from ELISA. All binders were further analyzed by DNA-sequencing and tested for their specificity by western blotting. Sequencing result showed that all binders have 93% homology with chicken immunoglobulin gene, and it was found that there have high variations in CDR region. For western blot analysis, binder 1 was specifically bind with H5 antigen.