Improvement of the stability and robustness of an Enzyme Linked Immunosorbent Assay (ELISA) for Foot and Mouth Disease (FMD) using mutated viral 3ABC non-structural recombinant protein as antigen

Foot and Mouth disease is a highly contagious viral disease of ruminants and swine. It constitutes an important threat to livestock production and trade worldwide. Its control would make a major contribution to international trade in animals and animal products. In developed countries, this disease has been eradicated after mass vaccinations and the implementation of a strict stamping out policy involving the culling of susceptible animals in the infected zones. However, in developing countries where it is impossible to implement such a policy as it is highly costly, FMD is still endemic and thus poses a constant threat to other countries where the disease has been eradicated, but animal are highly susceptible to infection. In such regions, control of FMD relies on the vaccination of animals. A more cost effective control policy would be to apply a systematic vaccination campaign combined with the identification of true infected and their selective elimination. The development of a test which can enable the differentiation of infected animals from those which have been vaccinated has made possible the implementation of such a strategy.

At the Animal Production Unit (APU) of the FAO/IAEA Agriculture and Biotechnology Laboratory in Seibersdorf an indirect ELISA (*iELISA)* and a competitive ELISA (*cELISA)* were developed for the ***D***ifferentiation between FMD ***I***nfected and ***V***accinated ***A***nimals (DIVA). The ELISA are based on the use of the FMD virus (FMDV) non-structural protein (NSP) 3ABC. This protein is not a part of the virus particle and is found only in cells where active FMDV multiplication has taken place. Since the current FMD vaccines are prepared from purified and killed virus, theoretically animals which are inoculated with non contaminated 3ABC protein will not develop antibodies against this protein as opposed to animals which have experienced the FMDV multiplication. Such differentiation between infected and vaccinated animals will be important in the future in the control of FMD: culling only infected animals instead of the total stamping out policy.

The NSP 3ABC is highly susceptible to enzymatic degradation because the C part of the protein is a protease, thus it induces its “self-digestion”. This characteristic might affect the repeatability and robustness of the test. This issue has been addressed in APU by producing a recombinant NSP 3ABC where the genes have been engineered in such a way as to inactivate the protease function and the proteolysis cleavage sites. For that purpose two DNAs (3ABC site_pro_mut and 3ABC_mut_optG) corresponding to the FMDV 3ABC gene were synthesised but including the required mutations for their inactivation. The codons of one of the mutated genes were optimized in order to improve production of the recombinant protein in insect cells. A third gene, corresponding to the normal gene, was also synthesized to represent the wild-type protein

(3ABC_WT_Ge) as a control. The various DNAs were inserted into the baculovirus by recombinant technology and their corresponding proteins were expressed in insect cells.

To examine the stabilizing effect of mutations in the gene, a long-term stability study with the different recombinants was carried out over a period of 40 days. They were incubated at 4 different temperatures: 4°C, 37°C, -20°C, -80°C. Aliquots of the different samples were made at day 1, 2, 3, 11, 25 and 40 after incubation and by gel protein electrophoresis followed western blot using a monoclonal antibody anti FMD NSP produced in APU.

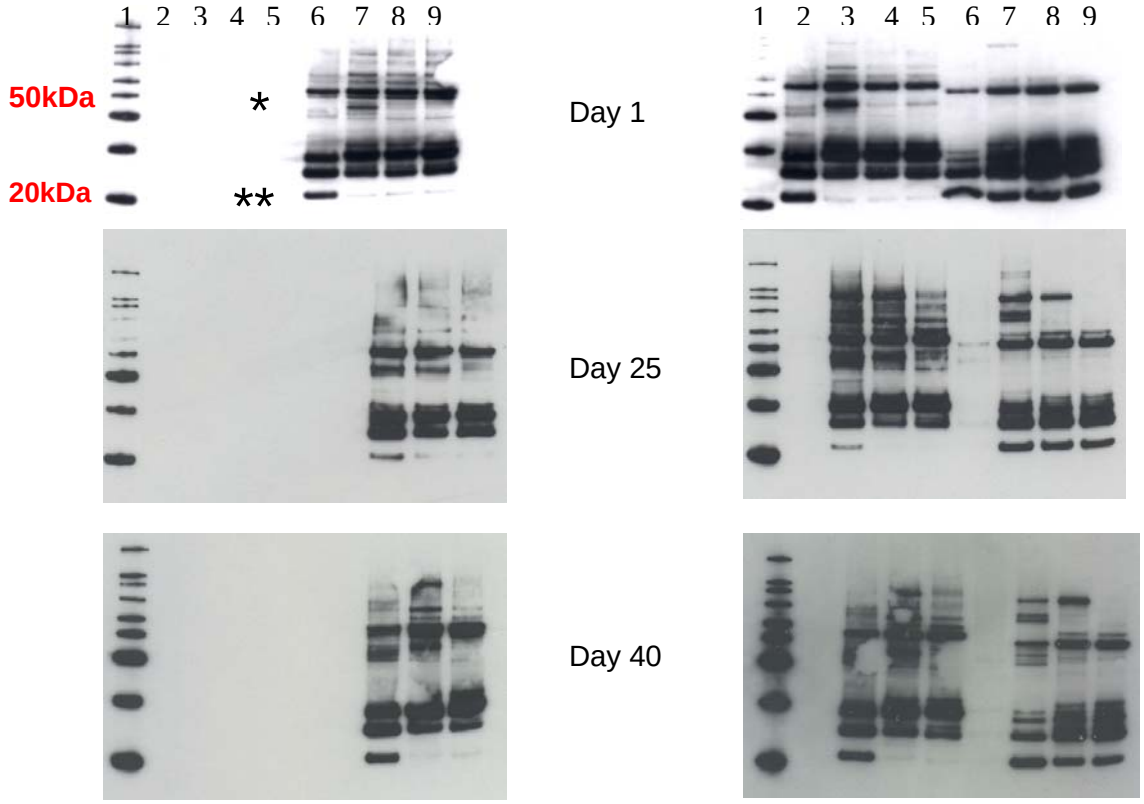

Figure 1: ***Left:*** *Western blot results after 1, 25, 40 days of incubation. Lane 1: Protein Molecular Weight Marker MagicMark XP; lane 2: SF21 insect cells, non-infected/+37℃; lane 3: SF21 insect cells, non-infected/+4℃; lane 4: SF21 insect cells, non-infected/-20℃; lane 5: SF21 insect cells, non-infected/-80℃; lane 6: 3ABC_mut_optG/+37℃; lane 7: 3ABC mut_optG/+4℃; lane 8: 3ABC_mut_optG/-20℃; lane 9: 3ABC_mut_optG/-80℃; *: 3ABC NSP 50kDa, **: 20kDa degraded protein.* ***Right:*** *Western blot results after 1, 25, 40 days of incubation. Lane 1: Protein Molecular Weight Marker MagicMark XP; lane 2: 3ABC_site_pro_mut /+37℃; lane 3: 3ABC_site_pro_mut /+4℃; lane 4: 3ABC_site_pro_mut /-20℃; lane 5: 3ABC_site_pro_mut /-80℃; lane 6: 3ABC_WT_Ge /+37℃; lane 7: 3ABC_WT_Ge /+4℃; lane 8: 3ABC_WT_Ge /-20℃; lane 9: 3ABC_WT_Ge / -80℃; *: 3ABC NSP 50kDa, **: 20kDa degraded protein.*

The results of this analysis confirm our hypothesis:

(i) The expression of the 3ABC protein in insect cells with the DNA incorporating the optimized codons gives more efficient protein production in insect cells, thereby ensuring optimal protein expression using the baculovirus expression system.

(ii) The mutated proteins are more stable than the wild type. Indeed, if stored at 4°C, -20°C and -80°C, the degradation of the wild type protein starts at day 1. But for the mutated proteins, the degradation is noted only from day 3 onwards when stored at 4°C. However, they are perfectly preserved when stored at -20°C and -80°C but the incubation at 37°C results in complete protein degradation for all samples used, mutated or wild type as well.

It is well known that insect cells are very rich in proteases. So the rapid degradation of the wild type 3ABC FMD NSP may be the result of their activities in combination to that of the self processing due to the proteolytic activity of the C part of the protein. By inactivating its function and its target proteolysis sites, the mutated protein is subject only to the degradation process of the insect cell proteases. To eliminate this possibility, the mutated NSP protein has been expressed in an *in vitro* bacterial *E. coli* expression system (the rapid translation system, -RTSystem). Previously it was noted that proteins with a histidine tag which are expressed in that system are easier to purify than the same tagged protein expressed in insect cells. The stability of this recombinant FMD NSP 3ABC protein and its evaluation in ELISA tests are being studied.