HOME-MADE DIAGNOSTIC KITS FOR HUMAN AND ANIMAL DISEASES : A DEVELOPING MARKET

Author: ***Ho Huynh Thuy Duong***
University of Natural Sciences, National University at HCMC, Vietnam
Tel: (84) 8 8350097 Fax: (84) 8 8350097
Email: hhtduong@hcmuns.edu.vn

Why the need of home-made diagnostic kits for hman and animal diseases ?

In Viet Nam, as in some other developing countries, socio-economic improvements, especially in urban areas, lead to increasing demand of high quality of life. This quality involves living environment, health care, food and clothes production. So high quality products and effective quality control become more and more important.

In the domain of health care, effective means of prevention, diagnosis and therapy are the aim of almost all medical researches. Vaccines, diagnostic kits and drugs production constitute a continuously growing market with quasi-unlimited potential. But, due to this enormous economic profit, products are chosen based on large demands. Some diseases affecting populations in developing countries do not fulfil financial criteria so do not benefit from interest of pharmaceutical firms. Another problem concerns the non-affordable cost of these products for a large part of these populations. Treatment of infectious diseases, the most important challenge of community health in developing countries, depends on accuracy and efficiency of diagnosis. Classical diagnosis, based on clinical, biochemical or immunological status, encounter problems of specificity, sensitivity and time consuming ; and in some cases, are not possible due to minute amounts of samples or inability to grow pathogens. Molecular methods for the detection of pathogens not only provide powerful tools for diagnostic as well as for epidemiological studies of infection.

Another field requiring efficient detection of pathogens is agriculture. Economic development of agriculture-based countries like Viet Nam involves development of plant culture and animal rearing. While traditional agriculture depends largely on experience and is totally submitted to fluctuations of natural conditions (flood, drought, …), large-scale productions require a greater control of the whole process – breeding, rearing and harvesting. Disease control is one of the key factors for a successful agriculture since diseases not only affect the yield but also products quality. Among infectious diseases in animals and plants, virus-causing diseases are of particular importance due to large and rapid extension of infectivity and lack of efficient treatment. Until now, farmers are obliged to use radical measures to prevent virus causing epidemics leading to great economic losses. An early and accurate detection of pathogens based on molecular methods will permit early intervention and reduction of damage. Imported molecular diagnostic kits for human and animal diseases do exist on the market. But their high cost limits their use, especially in agriculture.

Objectives of our project

Short-term objectives of the project consist of developing home-made diagnostic kits for human and animals. This aspect includes laboratory researches and production systems establishment.

Long-term objectives is to contribute to the promotion of regional diagnostic centers development by technology transfer, technical advisory and products supply.

Methods

Let take two examples, one diagnostic kit for a shrimp disease caused by WSSV (White spot syndrome virus) and another for viral hepatitis.

Laboratory researches

World aquaculture production continues to grow fast and is now contributing over 25% to the global supply of aquatic food products. According to official FAO estimates, world production of farmed shrimp in 1996 had a value of more than USD 6000 million. About 80% of the world's farmed shrimp are produced in Asia. Shrimp disease of which White spot syndrome (WSS) is one of the most serious, has emerged as one of the key issues affecting sustainability of shrimp farming worldwide. Outbreaks of WSS have led to serious mortalities among populations of cultured shrimp in several Asian countries including Viet Nam. Devastating effects of WSS on the shrimp farming industry were huge with losses in the order of billions of US dollars. In Viet Nam, a serious outbreak of viral disease occurred in late 1993 and the estimated loss was of 5,219 mt of shrimp. In 2002, rearing surface infected was estimated to about 30-60%.

Traditional diagnosis of WSSV are mainly based on non-specific clinical signs of shrimp. Microscopic examination of cytopathologic effects, the presence of inclusion bodies, are usually used to detect WSSV. But clinical as well as histological effects of WSSV can only be detected in advanced stages of infection for which it exists no measures.

Therefore, several research groups concentrated their efforts on the development of molecular methods to identify the virus. These methods include dot blotting, *in situ* hybridization, immunoassays and especially PCR which is a very sensitive and specific method. WSSV diagnostic PCRs are now becoming widely available, some are in kit form and have been used to screen spawners, shrimp larvae, or to monitor cultured shrimp populations. But the cost of these imported products are usually high, for example :

We set up PCR-based protocol for detection of WSSV in shrimp. DNA is first extracted from samples, amplified by PCR and amplicons are visualized in gel electrophoresis. Sensitivity and specificity of the protocol are determined and compared with those of protocols previously reported. The last step involves evaluation of the protocol through its application on shrimp samples collected from rearing ponds. Results obtained showed that our protocol can be used to screen adult shrimp as well as post-larvae with high sensitivity and specificity.

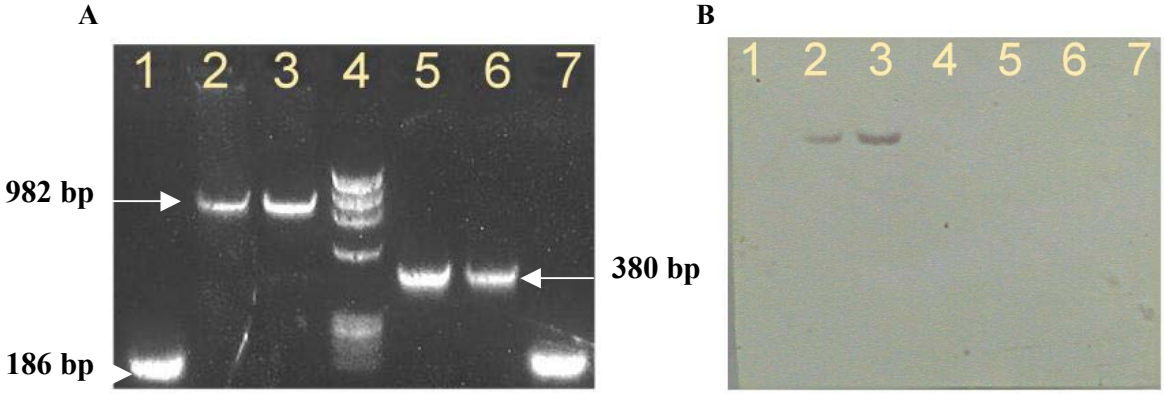

Fig.1 : Agarose gel electrophoresis (A) and Southern blot (B) analysis of WWSV, YHV and MBV

ΦX174-*Hae* III (4)

Amplicons from YHV RNA (1, 7) ; WSSV DNA (2, 3) ; MBV DNA (5, 6)

Viral hepatitis is a systemic infection involving hepatic cell necrosis and hepatic inflammation. There is at least five distinct viruses A, B, C, D and E which are causative agents of hepatitis. HCV (Hepatitis C Virus) and HBV (Hepatitis B Virus) are major causes of chronic hepatitis and are associated with cirrhosis, hepatic failure and hepatocellular carcinoma. Diagnosis based on clinical symptoms did not distinguish different hepatitis viruses. Serological tests are more specific but do not provide direct identification of HBV and HCV genome. Furthermore, because of the period between infection and appearance of anti-HCV is 22 weeks, these tests are not optimal for the detection of acute HCV infection. Concurrent infections with both HCV and HBV are increasingly recognized in patients with both acute and chronic hepatitis. Therefore, concomitant amplification and detection of HBV and HCV may simplify blood testing by reducing the number of tests required. In VietNam where HBV infection is endemic, effects of coinfection in the development of hepatic diseases have to be seriously investigated.

We set up a protocol for simultaneous detection of HCV and HBV genomic sequences based on PCR which is a very sensitive and specific technique. HCV RNA and HBV DNA are co-extracted from serum samples, co-amplified and amplicons are detected by agarose gel electrophoresis. This test provides diagnostic tool as well as means for epidemiological studies of co-infection with the two viruses in the population.

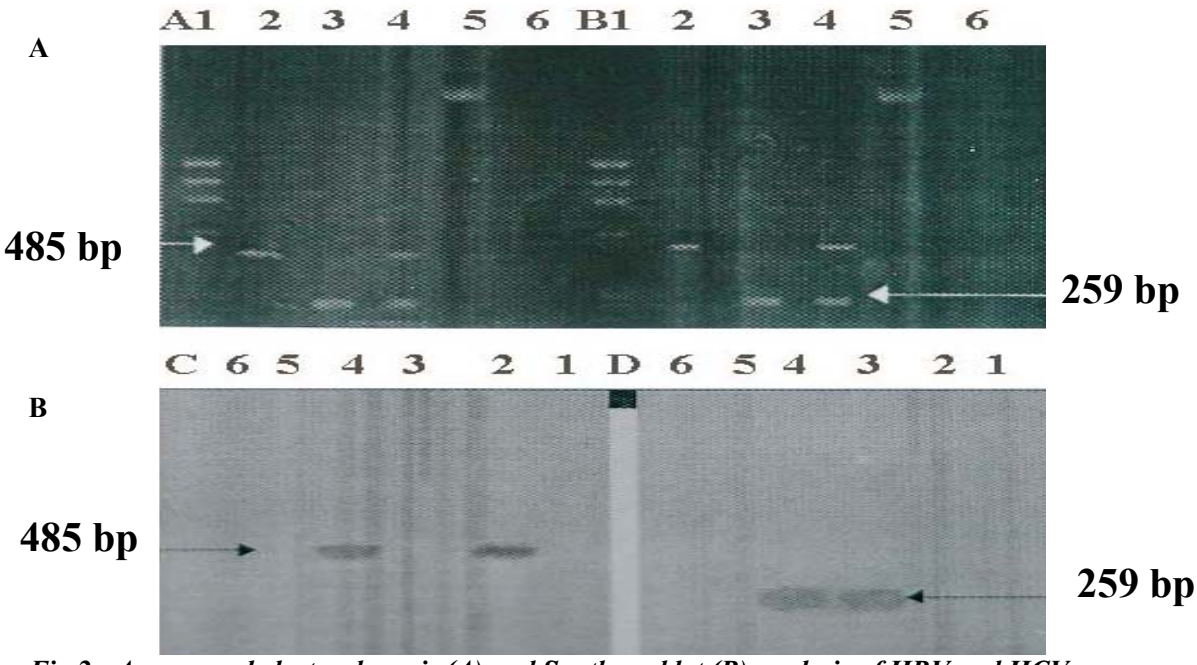

Fig.2 : Agarose gel electrophoresis (A) and Southern blot (B) analysis of HBV and HCV
ΦX174-*Hae* III (1) ; amplicons from HBV DNA (2) ; HCV RNA (3) ; combined HBV DNA-HCV RNA (4) ; $pGEM_3$ (5) ; Dengue Virus RNA type 3 RNA (6)

Interaction with consumers

Our researches are performed in response to the requests of organizations such as the Institute of Aquaculture for WSSV detection and the Hoa Hao Medical Center for Diagnosis in Ho Chi Minh City for the simultaneous detection of HBV-HCV. These organisms provide samples along with clinical diagnostic and informations about patients. They are or will be users which evaluate our results and aid in the improvement of our work.

The detection protocol for WSSV in shrimp has been modified several times to become more easy-handling and more refractory to contamination in routine diagnosis. Complementary RT-PCR based protocols were set up to simultaneously detect WSSV and YHV (Yellow Head Virus) or WSSV and MBV (Monodon Baculovirus) in response to demands of farmers.

Based on suggestion of consumers, HBV and particularly HCV typing methods were added to detection protocols to facilitate the deployment of appropriate treatment.

In both cases, continuous searches for cheaper materials are performed in order to lower product cost.

Training and technique transfer are until now realized through individual and group training. Technicians were asked to participate in workshop or theoretical courses at the University for acquisition of basic knowledge related to molecular diagnosis.

Results

We have elaborated two set of test kits : the first set concerns detection and typing kits for HBV, HCV separately and simultaneously, the second consists of detection kits for WSSV, YHV and MBV also separately and simultaneously. These kits are used to detect pathogens in a number of shrimp and clinical samples and showed high sensitivity and specificity.

Fig. 3 : Detection kit for combined infection with WSSV and YHV

These starting products will contribute to establish confidence of consumers in home-made kits. By making efforts to maximally lower the product cost, we try to promote the consumption of these products in place of imported kits.

Furthermore, training and technique transfer along with diagnostic kits supply will contribute to the establishment of some regional diagnostic laboratories and centers which will efficiently serve local economic and social development.

Networking is undertaken with the Institute of Biotechnology, the Institute of Aquaculture, Cho Ray Hospital to share experience and up-dated informations in research and applications.

Conclusions

The market for molecular diagnostic kits is a growing field in Vietnam.

Development of this market includes the development of theoretical knowledge and practical skills based on research and networking and formation of specialized personnel as well as kits production and supply.

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