CHAPTER I

INTRODUCTION

Hepatitis B virus infection is worldwide human health problem that result in acute and chronic hepatitis, cirrhosis, and the development of hepatocellular carcinoma (1). The serological of diagnosis of HBV infection is based on tests of HBsAg, anti-HBs, anti-HBc, HBeAg and anti-HBe. The different stages of infection between acute, chronic or post hepatitis B, are showed by specific patterns of these markers. The current blood donors screening of HBV infection is HBsAg. However, some data reported donors with seronegative for all HBV markers but positive for HBV DNA. Two of 34 Taiwanese blood donors lacking HBV markers, ALT elevation, and HBV DNA in dot blot hybirzation were positive for HBV DNA in PCR (2). Donor seronegative for all HBV markers but positive for HBV DNA in PCR have been identified and shown to harbor infectious that cause acute hepatitis and seroconversion in chimpanzees (3). There were data reported that up to 40% of individual with anti-HBc were carriers of HBV (HBV DNA positive but HBsAg negative), who may transmit hepatitis B when donating blood (4,5). The sequencing of the entire s-gene of HBV isolates from 14 donors with anti-HBc alone was determined and compared them to the entire s-gene of HBV isolates from HBsAg positive carrier. It was found that mutations in the s-gene were rather frequent. Since the "a"-determinant was more variable in solely anti-HBc positive isolates than in HBsAg control, this could well be reason the possible failure of commercial assays in detecting the surface antigen of these HBV carriers (6).

Hepatitis C virus HCV is the principal causative agent of post-transfusion and parenterally transmitted non-A, non-B hepatitis (7-8). The serological screening for HCV infection is based on detection of anti-HCV antibodies by immunoassay. Although the sensitivities of the anti-HCV assay have improved over recent years, a window period persists between infection and detectable seroconversion (9). One-third of HCV seroconversions take place as early as 2 weeks after infection (10), HCV antibodies may develop as late as 6 months after infection (11,12). Thus, anti-HCV blood donated during the infectious window periods may escape the blood safety-screening procedures. Reduction of the window period can be achieved by

assays targeting viral nucleic acids. It has been demonstrated that polymerase chain reaction (PCR) assay reduce the window period by 59 days for HCV infection (13,14).

Detection of viral nucleic acids by PCR might increase blood transfusion safety through the detection of recently infected blood donors during the preserocoversion window period. However, Individual screening is difficult to apply, because of technical and financial constraints. Pooling assays for the detection of multiple human viruses would decrease the cost of PCR-base screening (15).

One major concern as to the reliability of PCR is that it may generate false negative results, which can be due to a variety of factors, including presence of contamination inhibiting polymerase activity in the sample to be analyzed or simply mistakes in setting up the PCR. In a normal non-nested PCR protocol, this problem can be overcome by using low-stringency PCR [negative results are ascertained by the absence of non specific cellular bands (16)] or –especially when cellular material is to be analyzed-by co amplifying cellular genes (17). A very elegant control is the addition of different size internal control strand sharing the primer recognition sequences with specific template (18-20). Studies by Li, X. et al described the construction of a control template for a competitive nested primer PCR of the HIV-1 gag region that allows detection of false negative results when human immuno-deficiency virus type 1 (HIV)- infected cell are to be analyzed with nested primer PCR (21).

Therefore, the purpose of this study is to develop multiplex PCR for detection of both HCV RNA and HBV DNA in pooled specimen and to develop internal control for nested primer PCR of HBV DNA that allows excluding of false negative result.