

## **CHAPTER V**

### **RESULTS**

#### **Part I. Development of multiplex HBV DNA/HCV RNA PCR for the detection of HBV DNA and HCV RNA**

In this study, multiplex PCR method was developed to detect HBV DNA and HCV RNA in pooled specimen using oligonucleotide primers from HBV precore and core region and HCV 5' non coding region. The PCR products of HBV DNA and HCV RNA could be detected at 266 bp and 139 bp, respectively, by electrophoresis in 1.5% agarose gel (Fig. 10). It was found that HBV and HCV positive mixed serum gave two separated band of HBV and HCV.

#### **Part II. Detection of sensitivity and limit of detection of multiplex HBV/HCV PCR**

Sensitivity of the multiplex HBV DNA/HCV RNA PCR for detection of HBV DNA and HCV RNA were determined by using various dilutions of known HBV DNA serum control consisted of approximately 196,294, 19,629, 1,962, 981, 490 and 196 copies/ml (containing the copies per assay were 3,925, 392, 39, 19, 9, and 3 respectively) and various dilutions of known HCV RNA serum control consisted of approximately 360,480, 36,084, 18,024, 9,012, 4,506 and 450 copies/ml (containing the copies per assay were 7,209, 720, 360, 180, 90, and 9 respectively) (Figs. 11, 12). The method could be detected HBV DNA and HCV RNA at concentration approximately 196 copies/ml (3 copies/assay) and 450 copies/ml (9 copies/assay), respectively.

The percentages of limit detection in 10 replicated extraction of diluted samples by multiplex HBV DNA/HCV RNA PCR were studied. From the diluted samples of HBV DNA at concentrations of 196,294, 19,629, 1,962, 981 and 196 copies/ml, the percentage of detection was 100, 100, 90, 80, and 40, respectively (Fig.13). From the diluted samples of HCV RNA at

concentrations of 360,480, 36,084, 18,024, 9,012, 4,506 and 450 copies/ml, the percent detection was 100, 100, 100, 100, 100 and 80, respectively (Fig. 14).

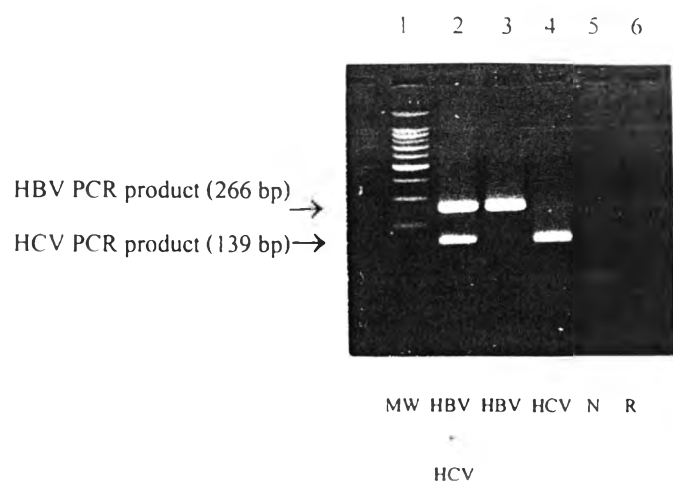


Figure 10. Agarose-gel electrophoresis showing the PCR products of multiplex HBV DNA/ HCV RNA PCR. (lane 1, molecular size marker, 100 bp ladder; lane 2, DNA and RNA from a mixture of sera from patient with HBV and HCV positive; lane 3, HBV DNA from serum of patient with HBV positive; lane 4, HCV RNA from serum of patient with HCV positive; lane 5, negative control (normal plasma); lane 6, reagent control).

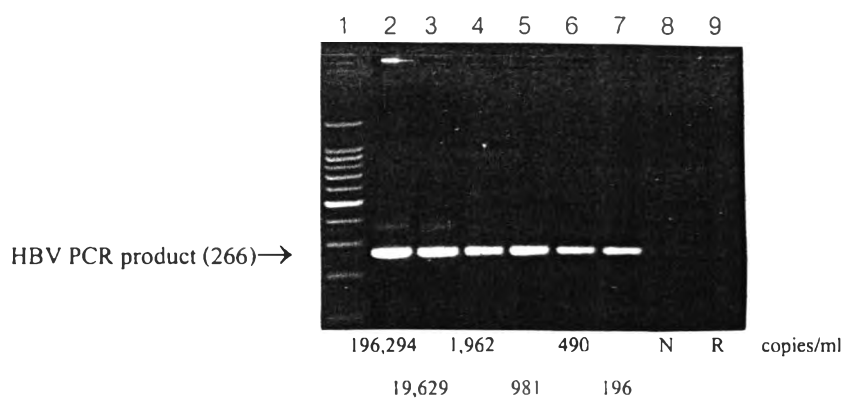


Figure 11. Agarose-gel electrophoresis showing the sensitivity of multiplex HBV DNA/ HCV RNA PCR for HBV DNA detection in various dilutions. (lane 1, molecular size marker, 100 bp ladder; lane 2, HBV DNA 196,294 copies/ml; lane 3, HBV DNA 19,629 copies/ml; lane 4, HBV DNA 1,962 copies/ml; lane 5, HBV DNA 981 copies/ml; lane 6, HBV DNA 490 copies/ml; lane 7, HBV DNA 196 copies/ml; lane 8, negative control; and lane 9, reagent control).

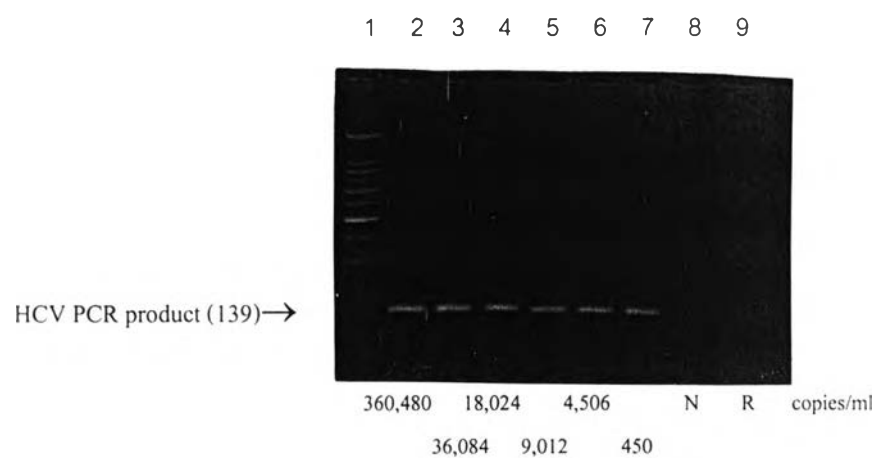


Figure 12. Agarose-gel electrophoresis showing the sensitivity of multiplex HBV DNA/ HCV RNA PCR for HCV RNA detection in various dilutions. (lane 1, molecular size marker, 100 bp ladder; lane 2, HCV RNA 360,480 copies/ml; lane 3, HCV RNA 36,048 copies/ml; lane 4, HCV RNA 18,024 copies/ml; lane 5, HCV RNA 9,012 copies/ml; lane 6, HCV RNA 4,506 copies/ml; lane 7, HCV RNA 450 copies/ml; lane 8, negative control and lane 9; reagent control).

### HBV DNA positive control

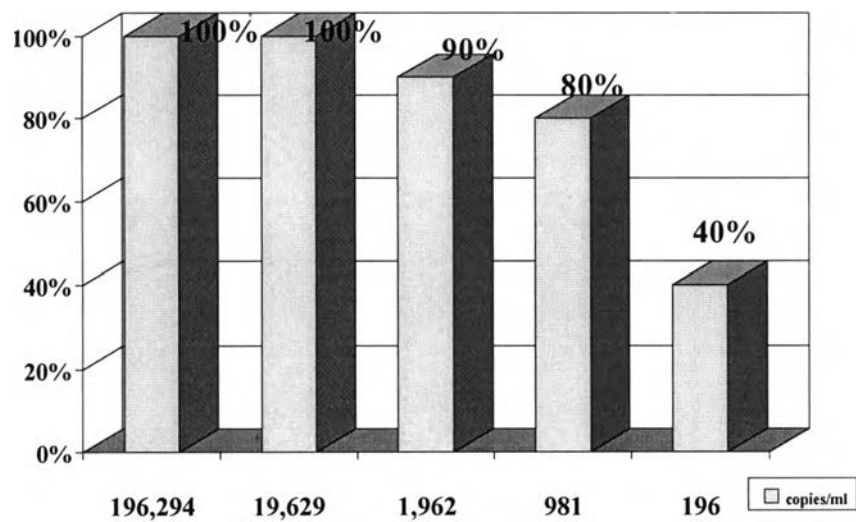


Figure 13. Graph showing the percentage of limit detection of multiplex HBV DNA/HCV RNA PCR for detection of HBV DNA by using various concentrations: 196,294, 19,629, 1,962, 981 and 196 copies/ml. The percentage detection was 100, 100, 90, 80, and 40, respectively.

### HCV RNA positive control

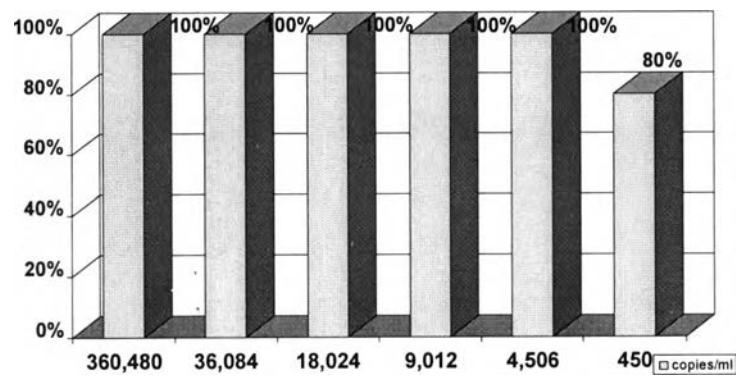


Figure 14. Graph showing the percentage of limit detection of multiplex HBV DNA/HCV RNA PCR for detection of HCV RNA by using various concentrations: 360,480, 36,048, 18,024, 9,012, 4,506 and 450 copies/ml. The percentage of detection was 100, 100, 100, 100, 100 and 80, respectively.

### **Part III. Construction of the internal control template for nest primer PCR of HBV DNA**

The amplified product of the designed HBV PCR internal control as shown in Fig. 9, was 200 bp (Fig. 15). The calculation of copy numbers of internal control product was approximately  $5.07 \times 10^9$  copies / $\mu$ l. The internal control was added into each reaction tube containing the HBV template for excluding false-negative result. When the internal control amplified product was seen in the individual run, it indicates that the result is reliable and interpretable. If there was no the product band of both internal control and target, this suggests that the amplification was failed and the result can not be interpreted.

The optimal copy numbers of internal control for the individual HBV PCR run was assessed in a wide range of concentrations of HBV wild type template (392, 196, 39 and 3 copies) and internal control (250, 25 and 2 copies). As shown in Fig. 16, the optimal internal control concentration was 25 copies.

The internal control was provided for the PCR reaction, twenty-five copies of internal control were added into a reaction tube containing the specific sample. The PCR was performed using multiple HBV DNA/HCV RNA PCR as shown in Fig. 17.



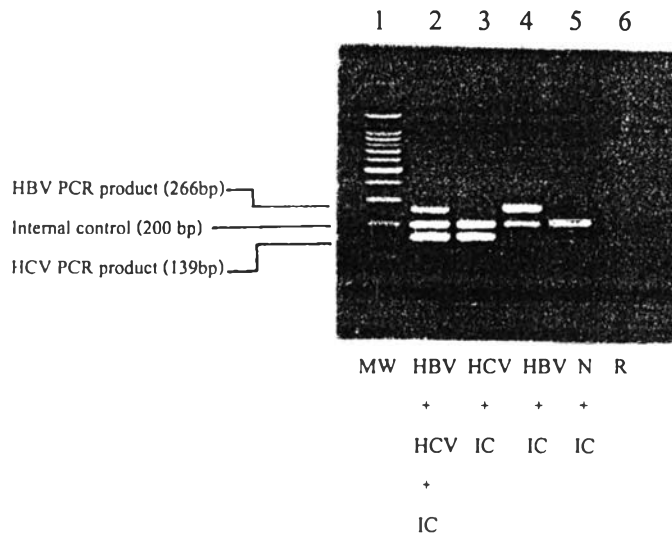


Figure 15. Agarose-gel electrophoresis showing PCR product of HBV PCR internal control at 200 bp, HBV PCR product at 266 bp, and HCV PCR product at 139 bp (lane 1, molecular size marker, 100 bp ladder; lane 2, DNA and RNA from a mixture of serum from patients with HBV and HCV positive; lane 3, HCV DNA from serum of patient with HCV positive; lane 4, HBV RNA from serum of patient with HBV positive; lane 5, negative control (normal plasma); lane 6, reagent control).

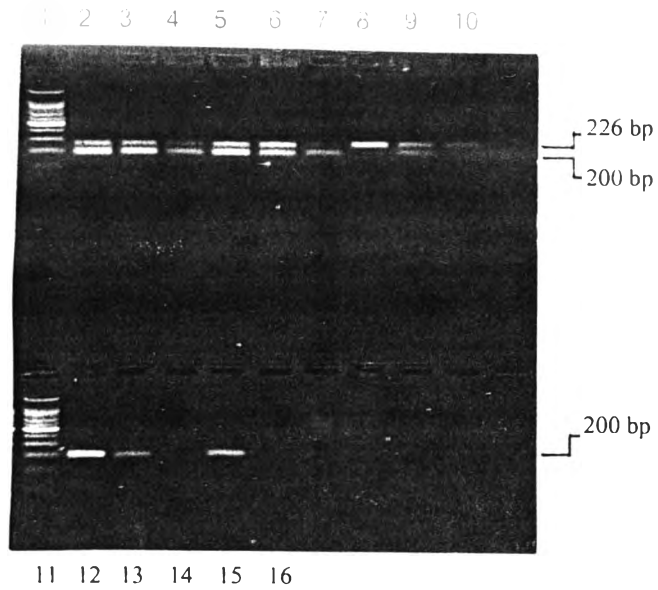


Figure 16. Agarose-gel electrophoresis showing the optimal copy numbers of internal control (IC). The individual HBV PCR run was assessed in a wide range of concentrations of HBV wild type template and internal control (IC).

lane 1, 11, molecular size marker, 100 bp ladder:

lane 2, wild type HBV template 392 copies and IC 250 copies;

lane 3, wild type HBV template 196 copies and IC 250 copies;

lane 4, wild type HBV template 39 copies and IC 250 copies;

lane 5, wild type HBV template 392 copies and IC 25 copies;

lane 6, wild type HBV template 196 copies and IC 25 copies;

lane 7, wild type HBV template 39 copies and IC 25 copies;

lane 8, wild type HBV template 392 copies and IC 2 copies;

lane 9, wild type HBV template 196 copies and IC 2 copies;

lane 10, wild type HBV template 39 copies and IC 2 copies;

lane 12, wild type HBV template 3 copies and IC 250 copies;

lane 13, wild type HBV template 3 copies and IC 25 copies;

lane 14, wild type HBV template 3 copies and IC 2 copies;

lane 15, IC 2 copies;

lane 16, reagent control.

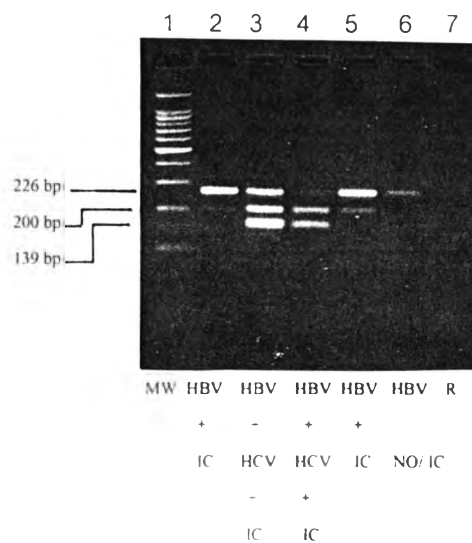


Figure 17. Agarose-gel electrophoresis showing the addition of optimal copy numbers of internal control for detection of both HBV DNA and HCV RNA using multiplex HBV DNA/HCV RNA PCR. (lane 1, molecular size marker, 100 bp ladder; lane 2, HBV DNA positive + IC 25 copies; lane 3, HBV DNA positive + HCV RNA positive + IC 25 copies; lane 4, HBV DNA positive (39 copies) + HCV RNA positive (90 copies) + IC 25 copies; lane 5, HBV DNA positive + IC 25 copies; lane 6, HBV DNA positive (39 copies), no IC; and lane 7, reagent control).