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SIMPLE DETECTION OF HEPATITIS C VIRUS USING ^{125}I-2'-DEOXYURIDINE TRIPHOSPHATE AND GAMMA COUNTER

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Objectives Hepatitis C Virus(HCV) is the major cause of post transfusion and sporadic non A, non B hepatitis. Current infection of HCV can be detected by PCR method. Using PCR, it has been possible to detect HCV viremia prior to immunological sero-conversion and to detect fluctuation of viremia in antibody-positive chronic HCV patients undergoing theraphy with interferon. In this study, we established the simple method to detect HCV DNA by incorporation of ^{125}I-deoxyuridine triphosphate(dUTP) into DNA during the PCR, and counted the radioactivity of PCR product by gamma counter. **METHODS** ^{125}I-2'-deoxyuridine 5'-triphosphate was prepared, and incorporated into DNA during PCR. dUTP was radiolabeled by the iododemercuration of 5-mercuri intermediate. Iododemercuration labeling was completed with 98% yield and the obtained product was incorporated into DNA without further purification. After incorporation, covalently bonded radioiodine substituent was remained stable during PCR procedure. HCV positive standard and positive patient sera in immunological assay were centrifuged. HCV RNA is isolated from by GTC(Guanidine Thiocyanate)and phenol/chloroform extraction method and synthesized complementary DNA by using reverse transcriptase. The ^{125}I-dUTP was incorporated into HCV C DNA during PCR. PCR product purified by fiber matrix column and counted by gamma counter. PCR products were electrophoresized, and autoradiography image obtained. **RESULTS** Amplified HCV DNA by ^{125}I-dUTP PCR obtained the band on the gel by electrophoresis and autoradiography at the same position. In patient sera, radioactivity of HCV positive sample was 8 times higher than HCV negative viremia sample. **CONCLUSION** We established HCV detection method using ^{125}I-dUTP. ^{125}I-dUTP PCR detection of HCV is convenient and reproducible.