Applicability of an oligonucleotide probe in radioisotope 32P-based dot blot hybridization for detection of hepatitis C virus in large sample numbers: a preliminary study

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Abstrak

Latar belakang: Penelitian ini bertujuan untuk merancang dan menganalisis pelacak oligonukleotida apakah dapat diterapkan dalam hibridisasi dot blot menggunakan radioisotop 32P untuk mendeteksi virus hepatitis C.
Metode: Sampel yang digunakan adalah 46 plasma darah. Plasma diekstraksi untuk mendapatkan RNA genom virus sebagai cetakan reaksi RT-PCR dan amplikon digunakan untuk nested PCR. Genom HCV berjumlah 24 diunduh dari GeneBank dan penderetan sekuen DNA dilakukan dengan Software Bio Edit versi 7.0.9.0. Pelacak oligonuklueotida dirancang berdasarkan daerah lestari genom HCV yang terletak pada sekuen internal di antara 2 primer yang digunakan pada nested PCR. Homologi oligonukleotida HCV dianalisis menggunakan teknik Blast di GeneBank. Radioisotop 32P digunakan untuk melabel oligonukleotida. Oligonukleotida berlabel diaplikasikan untuk produk nested PCR menggunakan metode hibridisasi dot blot. Konfirmasi hasil amplifikasi dan hibridisasi dot blot dilakukan menggunakan metode sekuensing DNA.
Hasil: Hasil analisis Blast menunjukkan homologi yang tinggi untuk HCV (100%). Hasil nested PCR menunjukkan tiga pola fragmen DNA. Tiga pola tersebut masing-masing adalah genotip HCV 1, 2, dan 3. Primer yang digunakan dalam nested PCR tidak spesifik dinyatakan dengan adanya tiga fragmen DNA sehingga sulit diinterpretasikan. Hasil hibridisasi dot blot menggunakan oligonukleotida yang didesain dalam penelitian ini menunjukkan intensitas dot yang tebal. Semua pola fragmen hasil nested PCR menunjukkan hasil positif dot blot. Hasil hibridisasi dot blot sesuai dengan hasil sekuensing DNA.
Kesimpulan: Pelacak oligonukleotida menunjukkan kriteria yang sangat memuaskan secara bioinformatika. Hasil hibridisasi dot blot menggunakan 32P menunjukkan intensitas dot yang tebal dan lebih mudah diinterpretasi dibandingkan dengan hasil nested PCR.
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Abstract
Background: This study aimed to design and analyze the applicability of an oligonucleotide probe in radioisotope 32P-based dot blot hybridization for detection of hepatitis C virus.
Methods: Forty-six of plasma samples were used. The plasma was extracted to obtain viral RNA genome as template for RT-PCR and the amplicon was used for nested PCR. Twenty-four HCV genomes were retrieved from GeneBank DNA sequence and alignment was performed by Bio Edit Software version 7.0.9.0. An oligonucleotide probe was designed based on a highly conserved region that is located on internal sequence between two primers used for nested PCR. Blast analysis on GeneBank was performed to obtain homology of the oligonucleotide for HCV. The oligonucleotide was then labeled with 32P and dot blot hybridization was applied for nested PCR products. DNA Sequencing was performed to confirm the amplicon and dot blot hybridization results.
Results: Blast analysis showed high homology (100%) for HCV. Nested PCR resulted in three patterns of

DNA fragments representing HCV genotypes 1, 2, and 3, respectively. The primers used in nested PCR were not specific and resulted in DNA fragments difficult to be interpreted. Dot blot hybridization using the designed oligonucleotide showed high intensity dots. All nested PCR fragments showed the dot blot positive. The dot blot results were in accordance with DNA sequencing that confirmed three patterns of DNA fragments as different HCV genotypes.

Conclusion: The oligonucleotide showed excellent bioinformatically criteria. 32P-based dot blot hybridization yielded high intensity dots and was easier to be interpreted than nested PCR assay.