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Rapid detection of common viruses using multi-analyte suspension arrays

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ABSTRACT

A method that uses specific oligonucleotide probes coupled to a specific array of fluorescent microspheres in multi-analyte suspension arrays was employed for the detection of common viruses, such as Herpes virus (HSV), Human papillomavirus (HPV) and Hepatitis B virus (HBV). Sixteen species-specific probes and 9 sets of specific primers were designed based on conserved sequences of these viruses in the GenBank database. Serial symmetric PCR, asymmetric PCR and multiple PCR assays were employed to evaluate the sensitivity, specificity and reproducibility of multi-analyte suspension arrays analyzed on a Luminex-100 analyzer instrument. The symmetric PCR amplification of four types of HSV, four types of HPV and HBV genotypes of B, C and D, combined with their corresponding species-specific probes and specificities were completely concordant with the results from a comparative sequence analyses. There was no significant difference in the median fluorescence intensity (MFI) value between symmetric PCR and asymmetric PCR when the viral DNA concentration was above 10^4 copies/test. Both PCR products were negative in the multi-analyte suspension arrays with viral DNA concentrations less than 10^3 copies/test. A multi-analyte suspension array is a flexible, high-throughput, relatively simple method for rapid identification of common viruses in the clinical laboratory.

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1. Introduction

A number of new infectious viral diseases such as SARS (Wang and Chang, 2004), avian flu (Jahangir et al., 2010) and influenza A H1N1 (Lambert and Fauci, 2010) have emerged, potentially causing serious public health security problems (Jones et al., 2008; Wimmer et al., 2009). It is not only possible that these viruses may re-appear, but it is also possible that new viruses may emerge. As a consequence, the ability to diagnose infection is of the utmost importance, and in-depth research into new viral pathogens using advanced detection methods is critical.

The ability to detect viruses improves continuously. Conventional detection methods include virus isolation and identification, electron microscopy, and virus neutralization tests (Olsvik et al., 1994). Identification by molecular biology techniques has increased rapidly and is used extensively in laboratory medicine. The basic technology, polymerase chain reaction (PCR), provides a rapid, sensitive and specific means of viral detection. With the development of PCR technology, a number of modified PCR methods emerged that are able to address issues that conventional PCR cannot do. For example, nested PCR has increased sample detection sensitivity and accuracy. Multiple PCR improves detection efficiency. Real-time quantitative PCR offers a "real-time, online" test that can distinguish viral infection in samples that conventional PCR techniques cannot do. Therefore, PCR and its modifications are sensitive, specific, accurate and economical (Roque-Afonso et al., 2004; Perrott et al., 2009; Blaise-Boisseau et al., 2010).

Multi-analyte suspension array is a new biochip technology (Zhu et al., 2008; Leblanc et al., 2009) that was commercialized by Luminex. Based on Luminex's XMAP technology, this method has been tested for the identification of bacteria and viruses in clinical applications (Borucki et al., 2005). It uses a liquid suspension array with up to 100 uniquely color-coded bead sets (Defoort et al., 2000). Each bead is labeled with a specific ratio (signature) of red and infrared fluorophores (excited by a red laser) to assign it a unique spectral address (region) and can be attached to different protein molecules (antibody or antigen) or probes.

The fluorescent microspheres can be coated with antibody or antigen or probe specific to a particular bioassay, allowing the capture and detection of specific protein molecules or PCR amplified products(the primer is labeled with biotin) from a sample (Naciff et al., 2005), and then a biotinylated antibody for a different epitope is added to the reaction system. This is detected with Streptavidin-R-Phycoerythrin, a reporter molecule that is excited by the green laser. The beads are drawn single file through a flow cell where the

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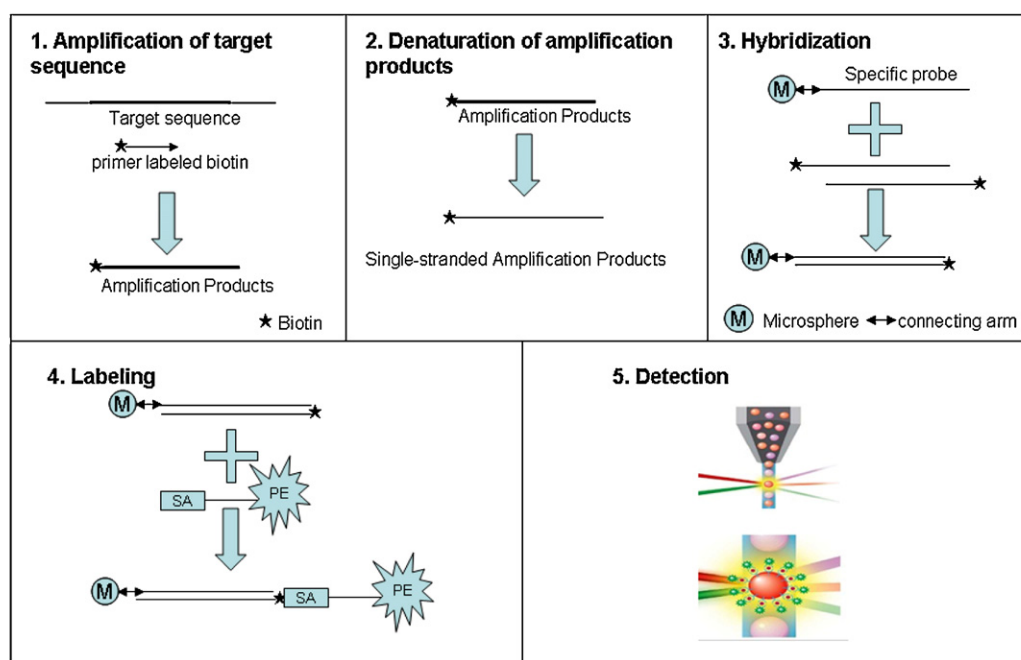

Fig. 1. Illustration of technical steps using the method described in this study. 1. DNA extracted from different virus samples is subjected to multiplex PCR using gene-specific primers labeled biotin.2. PCR amplified products are denatured at 95 °C to form single-stranded nucleic acids. 3. Denatured DNA is hybridized at 55 °C in a single multiplex assay. 4. The microspheres marked with each type-specific probe and complementary DNA complex are labeled with a mixture of phycoerythrin-streptavidin. 5. Detection and analysis of fluorescence emission are performed using the Luminex-100 analyzer instrument.

two lasers activate them. Using a dual-laser-based reader, the beads are analyzed in order to detect the specific analyte and the internal bead signature (region). These microspheres are suspended in a liquid phase and form multi-analyte suspension arrays that can detect several different target molecules in the same sample simultaneously (Fig. 1) (Deregt et al., 2006; Jiang et al., 2006; Ling et al., 2007; Croft et al., 2008; Drago et al., 2009). Compared with the current common experimental methods such as ELISA, RIA, Western blot and the single-analyte array, the multi-analyte array technology has the advantage of higher throughput, better flexibility, better specificity and a better signal to noise ratio (Croft et al., 2008; Ling et al., 2007).

In this study, based on the conservation and specificity of different viral genes, biotin-labeled symmetric PCR, asymmetric PCR and multiple PCR were carried out to amplify the herpes virus, four types of HPV and the S protein gene and polymerase P gene in the C region of hepatitis B virus. The amplification products were hybridized with specific probes connected to microspheres of the suspension array and detected those microspheres on the Luminex-100 analyzer instrument.

2. Materials and methods

2.1. Samples

Herpes virus DNA (HSV-1, HSV-2, CMV, and EBV) was detected from different specimens, including cerebrospinal fluid, sputum and throat swabs. The HPV genotyping detection specimens included vaginal discharge, leucorrhea, condyloma acuminatum warts and tissue explants. Hepatitis B virus genotyping detection specimen was serum. All specimens were the remaining samples reserved after clinical laboratory detection and were taken from outpatients and inpatients. Extracted DNA was detected by the fluorescence quantitative PCR method (DA AN molecular diagnostics company, Guangzhou, China); the positive samples with known viral load were divided into five aliquots and stored at −80 °C for future use.

2.2. Design of primers and probes

Conserved sequences of herpes virus, four types of HPV (6, 11, 16, and 18) and hepatitis B virus-specific genes were obtained from the Gene Bank and then the most conserved regions were selected after analyzing all the sequences by the CLUSTALW software. The specific primers were designed using Primer-Premier 5.0 (Table 1). The primers and probes with the best specificity were determined by BLAST sequence comparison. To enhance the hybridization specificity of each probe to its cognate target, each probe was designed to have at least three mismatched base pairs with its noncognate targets. Each probe was tagged at the 5′ end with an amino group, followed by a spacer of 18 thymines (Nucleotide Base), and finally was terminated 5′ with a specific probe; each of the PCR forward primers used in the multi-analyte suspension arrays detection was marked with biotin.

2.3. DNA template extraction

Sputum and throat swab were dissolved in normal saline solution. Condyloma acuminatum warts and tissue explants were placed in 4% sodium hydroxide solution and triturated using a metal bar. The samples were then vortex vibrated for 1 min, high-speed low-temperature centrifuged for 5 min (4 °C, 12000 × g/min); the supernatant was discarded, and 50 μl DNA extract solution (DA AN molecular diagnostics company, Guangzhou, China) was added to the pellet. 50 μl cerebrospinal fluid or serum samples were added to 50 μl DNA extract solution. The mixture of samples and DNA extract solution were then vortex vibrated for 1 min and heated at 100 °C for 10 min, high-speed low-temperature centrifuged for 10 min (4 °C, 12000 × g/min), and the supernatant was used as the viral DNA template.

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