Clearance of False-positive Antigen-Antibody Reactions of a Diagnostic Antigen Produced in *Escherichia coli* with Human Sera

Kap Soo Noh*, Jong Wan Kim, Suk Hoon Ha, Wang Don Yoo, Yeong Joong Jeon, and Hyun Su Kim

R & D Center of Cheil Jedang, Corp., 522-1 Dokpyong-Ri, Majang-Myon, Ichon-Si, Kyonggi-Do 467-810, Korea

Although many pharmaceutically useful proteins are produced in *E. coli* expression system, it is very rare for the system to be used in the production of diagnostic antigen due to a major problem, i.e., false-positive reaction of *E. coli* host-derived proteins contaminating purified diagnostic antigen with human sera. The N (nucleocapsid) protein of Seoul virus causing haemorrhagic fever with renal syndrome (HFRS) was produced in *E. coli* BL21 (DE3), and used for the detection of N protein-specific antibodies in human sera. Using the N protein as a diagnostic antigen of HFRS, the false-positive reaction was cleared by merely mixing the test sera with the extract of *E. coli* host strain not harboring expression plasmid.

Key words: diagnostic antigen, *E. coli*, false-positive reaction

INTRODUCTION

Although nucleic acid-based sensitive PCR (polymerase chain reaction) is available as a diagnostic method nowadays, it has some restrictions if the systems are to be used routinely. First, a pathogen has to be isolated from the serum of each individual and its genomic DNA or RNA should be prepared. Second, the diagnostic procedure is multi-step, time-consuming and inappropriate for the simultaneous testing of many samples. Thus many of infectious diseases are still diagnosed by the detection of pathogen-specific antibodies in the serum of an infected individual.

For the detection of pathogen-specific antibodies, diagnostic antigens of the pathogens should be prepared. Diagnostic antigens can be prepared either by the expression of some structural proteins using recombinant DNA technique or by cultivating the pathogens in some culture media or host cells. Among these two methods, recombinant expression systems provide a lot of advantages, such as avoiding of handling infectious material, ease of securing antigens in enough quantities at a lower preparation cost, and so on. Among many recombinant expression systems, *E. coli* system is most convenient for mass-production of foreign proteins. However, the antigen produced by recombinant *E. coli* system renders us a high probability of false-positive signals unless it is highly purified, since many individuals have high titers of antibodies against proteins of *E. coli*-origin. Here, we introduce a method to lessen a false-positive signal significantly when *E. coli*-produced protein is used as a diagnostic antigen with an example with N (nucleocapsid) protein of Seoul virus and human sera of haemorrhagic fever with renal syndrome (HFRS). Seoul virus is one of the major serotypes causing HFRS. Hantaviruses are rodent host-borne viruses belonging to the family *Bunyaviridae*. They are etiologic agents for two acute diseases, i.e., haemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS) [1,2]. N protein of hantavirus is a major viral antigen, and both anti-N IgM and IgG antibodies are detected in the sera at the onset of the symptoms of HFRS as well as HPS. Therefore anti-N antibodies are potentially useful targets for early diagnosis of HFRS and HPS.

MATERIALS AND METHODS

Cloning of Hantaviral Nucleocapsid Gene

Seoul viral cDNA pool was prepared from Seoul viral genomic RNA using random hexamers. The gene encoding Seoul viral N protein was amplified by two different PCR procedures. Primer 1 and 2 were used for the first PCR from the cDNA pool, and primer 2 and 3 were used for the second PCR from the first PCR product.

- Primer 1 (5'-TAGTAGTAGACTCCCTA-3')
- Primer 2 (5'-CCAGATCTATGGCAACTATGGAG-3'; *Bgl* II site underline)
- Primer 3 (5'-GGAATTCTTAGAGTTTCAAAGG-3'; *EcoR* I site underline)

The second PCR product was digested with *Bgl* II/*EcoR* I and inserted into *BamH* I/*EcoR* I sites of plasmid pET-3a. The resultant recombined plasmid was designated as a pET-sNP (Fig. 1).

Expression of Seoul Viral Nucleocapsid Protein in *E. coli*

The *E. coli* BL21 (DE3) carrying the T7 RNA polymerase gene under the control of *lacUV5* promoter [3] was transformed with pET-sNP (Fig. 1). The recombinant *E. coli* was cultured at 37°C in LB medium supplemented with 0.2% dextrose and 100 μg/mL ampicillin. The expression of N protein was induced by the addition of 1 mM IPTG when absorbance (600 nm) of the culture reached 0.5~0.7. The cells were cultured...
Purification of Nucleocapsid Protein

The cultured *E. coli* cells were disintegrated by sonication in 50 mM Tris/HCl (pH 8.0). The supernatant taken after centrifugation of the cell lysate was precipitated with 30% ammonium sulfate. The precipitate was re-dissolved in 40 mM KH₂PO₄/Na₂HPO₄ (pH 8.0) and chromatographed through a column of phenyl Sepharose. The flow-through fraction was taken and concentrated by ultrafiltration with a membrane filter having cut-off value of 50,000. The purified N protein was analyzed with SDS-PAGE and western blotting.

Western Blot Analysis for Reaction of N Protein with Human Sera

Western blot was performed according to the standard protocol [4]. Purified N protein was boiled in a loading buffer (5 mM Tris/HCl pH 6.8, 2% w/v SDS, 2.5% v/v glycerol, 0.005% w/v Bromophenol Blue, 2.5% v/v beta-mercaptoethanol) for 5 min. The samples were loaded on SDS-12% w/w polyacrylamide gels. Immunostaining was carried out with human sera and peroxidase conjugate of anti-human IgG, and by using coloring material 4-chloro-l-naphtol. For the pretreatment of human serum, 300 µg of *E. coli* extract was added in 10 mL of PBS (8 g NaCl, 0.2 g KCl, 1.44 g Na₂HPO₄, and 0.24 g KH₂PO₄ per liter of distilled water, pH 7.4) together with 10 mL of human serum. The mixture was used as a primary antibody for reaction with N protein transferred to nitrocellulose membrane. The specificity of the purified N protein was studied by immunodot blot using 20 healthy human sera, four of them showed positive signals. However, when the result was reconfirmed with western blot, all the positive signals were found to be false. There were no color bands at 50 kDa, the expected location of Seoul viral N protein. Meanwhile, color bands were observed at 90 kDa (Fig. 3). The protein of 90 kDa seems to be derived from cytoplasmic proteins of *E. coli* and to remain in the purified N protein. We tried to remove the contaminating protein by several methods such as ion-exchange, gel-filtration, and ultrafiltration, etc., but failed to clear the false-positive reaction completely by improving the purity of N protein.

We introduced another technique, i.e., pretreatment of human sera with *E. coli* extract, to remove antibodies showing antigen-antibody reaction with contaminating proteins of *E. coli*-origin in the purified N protein. This trick was very successful and false-positive reaction was cleared close to completion without affecting the sensitivity. The results of western blot analyses were shown in Fig. 4.