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Serodiagnosis Using Recombinant Nipah Virus Nucleocapsid Protein Expressed in *Escherichia coli*

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Nipah virus nucleocapsid (NiV-N) protein was expressed in *Escherichia coli* and purified by histidine tag-based affinity chromatography. An indirect immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) for human and swine sera and an IgM capture ELISA for human sera were established using the recombinant NiV-N protein as an antigen. One hundred thirty-three suspected patient sera and 16 swine sera were used to evaluate the newly established ELISA systems in comparison with the CDC inactivated-virus-based ELISA systems. For the human sera, the NiV-N protein-based indirect IgG ELISA had a sensitivity of 98.6% and a specificity of 98.4%, and the NiV-N protein-based IgM capture ELISA had a sensitivity of 91.7% and a specificity of 91.8%, with reference to the CDC ELISA systems. The NiV-N-based IgM ELISA was found to be more sensitive than the inactivated-virus-based ELISA in that it captured eight additional cases. For the swine sera, the two test systems were in 100% concordance. Our data indicate that the Nipah virus nucleocapsid protein is a highly immunogenic protein in human and swine infections and a good target for serodiagnosis. Our NiV-N protein-based ELISA systems are useful, safe, and affordable tools for diagnosis of Nipah virus infection and are especially fit to be used in large-scale epidemiological investigations and to be applied in developing countries.

Nipah virus (NiV) is a recently described zoonotic paramyxovirus that causes fatal encephalitis in humans (4, 17). In Malaysia and Singapore it has caused an outbreak of severe encephalitis among people in close contact with pigs. The outbreak was first noted in late September 1998, and by mid-June 1999, more than 265 encephalitis cases, including 105 deaths, had been reported in Malaysia, and 11 cases of encephalitis or respiratory illness, with 1 death, had been reported in Singapore. Later, it was noted that one-quarter of the survivors had residual neurological side effects (4, 17). In Malaysia, the outbreak of Nipah virus infection had a tremendous impact on the nation's economy and society in terms of the loss of human lives and the reduction, by more than 50%, of the number of live herds of swine (4).

Fruit bats (flying foxes) are believed to be the natural reservoir for Nipah virus (5, 8). Ecological changes associated with land use and animal husbandry practices appear most likely to be associated with the emergence of Nipah virus (8). Fruit bats exist in great numbers from India to Australia. The presence of Nipah virus has also been reported between 2001 and 2004 in Thailand, Cambodia, Bangladesh, and India (1, 3, 8, 10, 15, 18, 22). This demonstrates that Nipah virus represents a major health problem worldwide that could affect the economies of many countries. Therefore, there is a demand for rapid detection, as well as serological diagnosis, of Nipah virus in order to monitor the presence of the virus and its antibodies in humans and animals in high-risk areas.

Several enzyme-linked immunosorbent assay (ELISA) systems have been developed for serological tests after initial investigations of Nipah virus outbreaks. At the Centers for Disease Control and Prevention (CDC; Atlanta, Ga.), the approach has been not only to conduct an indirect ELISA for the detection of immunoglobulin G (IgG) but also to employ a capture ELISA system for the detection of IgM. These CDC ELISA methods were originally developed to detect Hendra virus antibodies and then transferred to Malaysia to be applied in response to the Nipah virus outbreak (4, 8). Another indirect ELISA was developed by the Australian Animal Health Laboratory utilizing a gamma-irradiated Nipah virus antigen (6, 8). Unfortunately, the production of immunological reagents for these assays requires high-security laboratories, which are limited to only a few countries worldwide.

NiV is a negative-sense, nonsegmented RNA virus that was first isolated from cerebrospinal fluid of human patients and classified in the family *Paramyxoviridae* under the new genus *Henipavirus*. Its genome encodes six structural proteins: the nucleocapsid (N) protein, phosphoprotein (P), matrix (M) protein, fusion (F) protein, glycoprotein (G), and large (L) protein (9). The N protein is the most abundant structural protein in the purified virion and has a calculated molecular mass of approximately 58 kDa. Nucleotide sequence analysis revealed that the N genes of NiV obtained from humans, pigs, cats, and dogs were identical to each other (4, 9).

Eshaghi, Tan, and colleagues reported that Nipah virus N (NiV-N) protein expressed in insect cells and *Escherichia coli*

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assembled into different types of structures of different lengths, including spherical, ring-like, and herringbone-like particles, under electron microscopy and that the recombinant N protein exhibited the antigenic sites and conformation necessary for specific antigen-antibody recognition (7, 16, 20). But since only a limited number of swine samples were tested, more studies are required to assess the use of the recombinant N protein in routine diagnosis, especially for human samples.

To evaluate the possibility of using recombinant NiV-N protein as a safe and affordable antigen for serodiagnosis of Nipah virus infection, we cloned the full-length N gene, expressed the N protein in *E. coli*, purified the N protein, developed N protein-based ELISA systems for the detection of Nipah virus infection, and evaluated them in comparison with the existing inactivated Nipah virus-based ELISA systems.

MATERIALS AND METHODS

Serum samples. One hundred thirty-three serum samples collected from human patients with suspected infections and 16 swine sera collected from pigs with suspected infections during the 1999 Nipah virus outbreak in Malaysia were analyzed in this study.

RNA extraction. A Nipah virus strain (Ma-JMR-01-98), isolated from the cerebrospinal fluid of an encephalitis patient, was propagated in the Vero-E6 cell line, maintained for 4 days at 37°C in Eagle's minimum essential medium supplemented with 2% fetal calf serum and 0.2 mM each nonessential amino acid. Upon observation of 80 to 100% cytopathic effect, the infected-culture supernatant was harvested and viral RNA was extracted from 140 μl of the infected-culture supernatant using the QIAamp viral RNA minikit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The extracted RNA was eluted in 60 μl of elution buffer and then used as a template for reverse transcription-PCR (RT-PCR).

Construction of recombinant plasmids. The Nipah virus N gene was amplified by RT-PCR as previously described (11, 23). PCR amplification was carried out using primers 5′-ATATGGATCCATGAGTGATATCTTT-3′ and 5′-GGAAGTCGACGTCACACATCAGCT-3′ to generate the full-length N gene of Nipah virus. Sense and reverse primers contained BamHI and SalI restriction sites (underlined), respectively. The 1.6-kb PCR-amplified DNA fragments were digested with BamHI and SalI, purified by a QIAEX II gel extraction kit (QIAGEN, Hilden, Germany), and subsequently cloned into the corresponding restriction site of the pQE30 vector (QIAGEN, Hilden, Germany). The insert of the recombinant plasmid was confirmed to be in frame by DNA sequencing. The expression construct, encompassing the full length of Nipah virus N protein (amino acids 1 to 532) with a vector-derived His tag (histidine hexamer) at the N terminus, was obtained. The resultant recombinant protein was designated NiV-N protein.

Expression and purification of the recombinant NiV-N protein. The recombinant NiV-N protein was expressed and purified as previously described (23). Briefly, *E. coli* strain XL-1 Blue was transformed by using the recombinant plasmid containing the NiV-N sequence and cultured at 30°C in LB medium containing 100 μg/ml of ampicillin. When the optical density (OD) at 600 nm of the culture reached 0.5, the expression of recombinant proteins was induced by the addition of 0.2 mM isopropyl-β-D-thiogalactoside (IPTG) for 3 h. After being harvested by centrifugation, the *E. coli* was washed in phosphate-buffered saline solution (PBS), resuspended in 10 mM PBS (pH 7.5)–500 mM NaCl, and frozen at −80°C. After being frozen and thawed three times, the cell suspension was sonicated for 2 min with an interval of 1 s between pulses and centrifuged at 30,000 × *g* for 15 min at 4°C. The supernatant was then applied to a Talon immobilized metal affinity column resin column (Clontech). After being washed with 10 mM PBS–500 mM NaCl containing 20 mM imidazole, the purified protein was eluted with 10 mM PBS (pH 7.5)–500 mM NaCl containing 250 mM imidazole. The protein solution was aliquoted and stored in a final concentration of 10% glycerol at −80°C for later use. The concentration of protein was determined by the Bradford method (2) using a Bio-Rad protein assay reagent kit, and the purity of the protein was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Western blot analysis. Western blot analysis was performed as described by Towbin et al. (21). Briefly, the recombinant protein was electronically separated in a 10% polyacrylamide gel before electrotransfer to a polyvinylidene difluoride membrane (Immobilon; Millipore) using a semidry electroblotter (Sartorius, Germany). The membrane was initially blocked with Blockace (Yokijirushi, Sapporo, Japan) overnight at 4°C and then reacted with a mouse antihistidine serum (1:1,000 dilution) or a patient serum (1:100 dilution) for 1 h at 37°C, followed by incubation with a peroxidase-conjugated rabbit anti-mouse IgG or goat anti-human antibody (1:1,000 dilution) for 1 h at 37°C. Finally, the reaction was visualized by dimethyl aminobenzidine (DAB) staining.

ELISA procedures using the recombinant nucleocapsid protein. To evaluate the possibility of using the recombinant NiV-N protein for the diagnosis of Nipah virus infection, an indirect IgG ELISA system for human and swine sera and an IgM capture ELISA system for human sera were developed using the NiV-N protein as the antigen. For all the ELISA procedures, 96-well Falcon immunoplates (Becton Dickinson) and 100-μl volumes of all reagents were used. The washing buffer comprised 0.01 M PBS with 0.1% (vol/vol) Tween 20 (PBS-T). Plates were washed five times with PBS-T between additions of different reagents. Plates were coated at 4°C overnight using 0.01 M PBS (pH 7.4) as the buffer diluent. All subsequent serum and reagent dilutions were done in 5% nonfat milk (Difco, Detroit, Mich.) in PBS-T. Incubations, except for the substrate, were done at 37°C for 1 h. After 100 μl of H_2O_2–ABTS (2,2′-azinodiethylbenzthiazoline sulfonic acid) substrate (Kirkegaard & Perry, Gaithersburg, Md.) was added to each well, the plates were incubated at 37°C for 30 min and then read spectrophotometrically, and the ODs at 405 nm were recorded. Each sample was tested in duplicate, and the mean OD for each sample was calculated. Reference sera were run every time the assay was carried out. The cutoff for the assay was twice the mean OD for the negative-control serum sample (positive/negative [P/N] ratio, ≥2).

Indirect IgG ELISA for human serum. The indirect IgG ELISA was performed by coating the plate with the purified recombinant NiV-N protein. The optimal concentration of recombinant NiV-N protein used to coat the microplates was determined by checkerboard titration with reference sera. The 96-well Falcon immunoplates were coated with 25 ng of recombinant NiV-N protein per well. The antigen was then removed from the wells by five washes with PBS-T. Human sera were diluted 1:400 and allowed to react with the antigen-coated wells. Bound IgG was detected with 1:30,000-diluted horseradish peroxidase-conjugated goat anti-human IgG (American Qualex, San Clemente, Calif.), followed by the addition of H_2O_2–ABTS substrate. ODs were recorded on a microplate spectrophotometer.

Indirect IgG ELISA for swine serum. Basically, the indirect IgG ELISA procedure for swine serum was the same as the procedure for human serum described above. The dilutions used for swine serum and horseradish peroxidase-conjugated goat anti-swine IgG (Cappel, ICN) were 1:100 and 1:1,000, respectively.

IgM capture ELISA for human serum. The Nipah virus-specific IgM test was done by first capturing the IgM from the serum with a goat anti-human IgM antibody adsorbed to the wells of the immunoplates and then allowing the captured IgM to react with the recombinant NiV-N protein. Captured NiV-N was detected with Nipah virus hyperimmune mouse ascitic fluid. Bound anti-N protein antibodies were detected by the addition of horseradish peroxidase-conjugated anti-mouse IgG, followed by H_2O_2–ABTS substrate. Optimal dilutions of all reagents were determined by checkerboard titration with reference sera. The actual procedures were as follows. Ninety-six-well Falcon immunoplates (Becton Dickinson) were coated with 100 μl of a 1:500 dilution of goat anti-human IgM (BioSource International) diluted in PBS (pH 7.4), and plates were coated at 4°C overnight. The plates were then washed five times with PBS-T, and patient serum samples were added at a 1:400 dilution in assay diluent. The patient samples were incubated on the plates for 1 h at 37°C, followed by washing. The antigen (purified recombinant NiV-N protein) was then added at a concentration of 125 ng/ml (in diluent), and the plate was incubated for 1 h at 37°C. The plates were washed, and a 1:4,000 dilution of anti-Nipah virus hyperimmune mouse polyclonal ascitic fluid (supplied by CDC) was added and incubated for 1 h at 37°C. The plates were washed, and a 1:5,000 dilution of horseradish peroxidase-conjugated goat anti-mouse IgG (BioSource, Camarillo, Calif.) was added and incubated at 37°C for 1 h. An ABTS peroxidase substrate (Kirkegaard & Perry Laboratories) was then added to wells after a wash. Plates were incubated in the dark for 30 min at 37°C, and ODs were measured at 405 nm. On all test plates, 1:400 dilutions of negative-control, weak positive-control, and strong positive-control serum samples were run simultaneously. Each sample was tested in duplicate, and the mean OD for each sample was calculated. The cutoff for the assay was twice the mean OD for the negative-control serum sample.

ELISA using Nipah virus-infected cell lysates. The indirect IgG ELISA for human and swine sera and the IgM capture ELISA for human serum using Nipah virus-infected Vero-E6 cell lysates were performed by following the CDC pro-

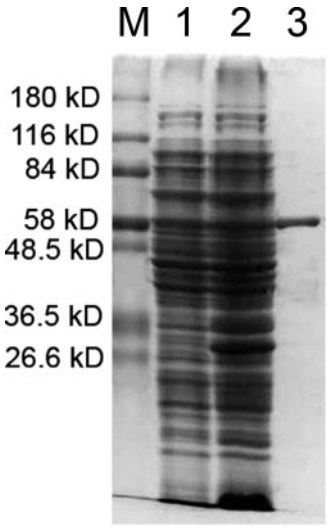

FIG. 1. Expression and purification of recombinant NiV-N protein. A recombinant plasmid containing the full-length Nipah virus N gene was transformed into *E. coli* XL1-Blue and induced with IPTG. *E. coli* cells were collected and dissolved in 10 mM PBS (pH 7.5)–500 mM NaCl. After sonication, the *E. coli* cell lysate was centrifuged and the recombinant protein was purified from the supernatant by use of a Talon immobilized metal affinity column. The *E. coli* cell lysate and purified recombinant protein were analyzed in a 10% SDS-PAGE gel and revealed with Coomassie brilliant blue staining. Lane M, protein marker (SDS-7B); lane 1, supernatant of sonicated *E. coli* cell lysate after centrifugation; lane 2, pellet of sonicated *E. coli* cell lysate; lane 3, purified recombinant protein.

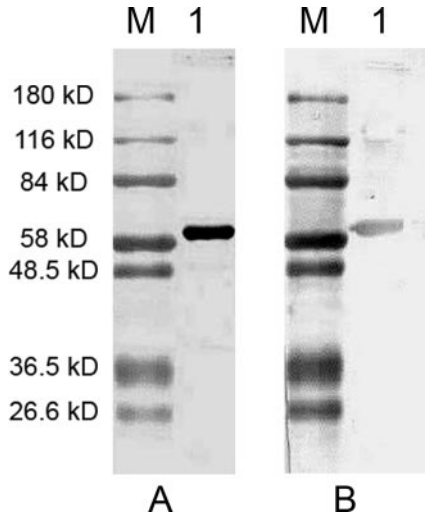

FIG. 2. Western blot analysis of purified NiV-N protein. A prestained protein marker and purified recombinant NiV-N protein were separated by SDS-PAGE and transferred to a polyvinylidene difluoride membrane. Each membrane was incubated with diluted mouse antihistidine serum or human patient serum followed by horseradish peroxidase-conjugated anti-mouse IgG or anti-human IgG (1:1,000 dilution) and the results visualized by DAB staining. (A) Reactivity of recombinant protein to mouse antihistidine serum. (B) Reactivity of recombinant protein to Nipah virus patient serum. Lanes M, protein marker (SDS-7B); lanes 1, purified NiV-N protein.

tocol (4, 8). The cell lysates and slurries of Nipah virus-infected and uninfected Vero-E6 cells were supplied by the CDC.

For the IgG ELISA, ELISA plates were coated with 1:2,000 inactivated-Nipah virus-infected Vero-E6 cell lysates at 4°C overnight. Plates were also coated with the uninfected Vero-E6 cell lysate antigen in order to determine the specific binding of the antibody to viral antigens. The antigen was removed from the wells by a wash with PBS-T. Sera here were diluted through four fourfold steps from 1:100 to 1:6,400 in 5% nonfat milk in PBS-T and were allowed to react with the antigen-coated wells. Bound IgG was detected with 1:30,000-diluted goat anti-human IgG (or 1:1,000-diluted goat anti-pig IgG for swine sera) conjugated to horseradish peroxidase, followed by color development with the ABTS substrate. The adjusted OD was calculated by subtracting the OD of the uninfected antigen-coated wells from that of the corresponding viral-antigen-coated wells. The cutoff value for the assay was defined as an individual OD of $\geq$0.2 or a sum of the ODs of the four dilutions of $\geq$0.95.

For the IgM capture ELISA, the procedure was the same as that described for the NiV-N IgM capture ELISA above, except that the sera here were diluted 1:100 and then fourfold through 1:6,400 in 5% nonfat milk in PBS-T; the antigen used was a 1:40-diluted inactivated Nipah virus-infected Vero-E6 cell slurry, and the control antigen was a 1:40-diluted uninfected Vero-E6 cell slurry. The cutoff value for the assay was defined as an individual OD of $\geq$0.1 or a sum of ODs of the four dilutions of $\geq$0.5.

RESULTS

Expression and purification of recombinant NiV-N protein. The Nipah virus nucleocapsid gene, encoding amino acid residues 1 to 532 of the full-length nucleocapsid protein, was amplified by RT-PCR and cloned into the BamHI and SalI sites of expression vector pQE30 in frame and downstream of the six-histidine tag. The sequence and reading frame of the N gene were confirmed by DNA sequencing of the recombinant plasmid. The recombinant protein was successfully expressed in *E. coli* by growth at a low temperature and induction with a low concentration of IPTG. Around 50% of the expressed protein was soluble. The NiV-N protein was purified from the supernatant by a Talon metal affinity column under natural conditions. Analysis of the purified recombinant protein by SDS-PAGE and Coomassie blue staining revealed a single protein band of 60 kDa, slightly larger than the predicted size of the N protein (58 kDa), possibly due to the His tag at the N terminus (Fig. 1). The identity of the recombinant NiV-N protein was further confirmed by a Western blot assay with the mouse antiserum to histidine and Nipah virus patient serum (Fig. 2).

Evaluation of recombinant NiV-N protein-based IgG and IgM ELISAs for humans. A total of 133 human serum samples collected from patients with suspected Nipah virus infection by the Institute for Medical Research, Kuala Lumpur, Malaysia, were used for the assessment of the NiV-N-based indirect IgG ELISA (NiV-N IgG ELISA) and IgM capture ELISA (NiV-N IgM ELISA), and these were compared with the inactivated-Nipah virus-infected cell-based ELISAs (CDC ELISAs).

Indirect IgG ELISA for human serum. For the indirect IgG ELISA, the genetically engineered NiV-N protein-based IgG ELISA and the virus antigen-based IgG ELISA from the CDC had a concordance rate of 98.5% (131 of 133). Of the 133 samples, 68 were positive and 63 were negative by both tests. One was positive by the NiV-N IgG ELISA but negative by the CDC IgG ELISA, and another sample was positive by the CDC IgG ELISA but negative by the NiV-N IgG ELISA. The sensitivity and specificity of the NiV-N IgG ELISA with regard to the CDC IgG ELISA were 98.6% and 98.4%, respectively (Table 1).

IgM capture ELISA for human serum. For the IgM capture ELISA, the NiV-N protein-based IgM ELISA and the CDC

TABLE 1. Sensitivity and specificity[a] of the NiV-N IgG ELISA with reference to the CDC IgG ELISA for human serum

CDC IgG ELISA results	NiV-N IgG ELISA results		Total
	No. positive	No. negative	
No. positive	68	1	69
No. negative	1	63	64
Total	69	64	133

[a] Sensitivity, calculated as (number of true-positive results)/[(number of true-positive results) + (number of false-negative results)] × 100, was 98.6%. Specificity, calculated as (number of true-negative results)/[(number of true-negative results) + (number of false-positive results) × 100, was 98.4%. Concordance, calculated as [(number of samples positive by both methods) + (number of samples negative by both methods)]/(total number of samples) × 100, was 98.5%.

IgM ELISA had a concordance rate of 91.7% (122 of 133). Of the 133 samples, 33 were positive and 89 were negative by both tests. However, eight samples were positive by the NiV-N IgM ELISA but negative by the CDC IgM ELISA, and three samples were positive by the CDC IgM ELISA but negative by the NiV-N IgM ELISA. The sensitivity and specificity of the NiV-N IgM capture ELISA with regard to the CDC IgM ELISA were 91.7% and 91.8%, respectively (Table 2).

In both the recombinant NiV-N protein-based indirect IgG ELISA and the IgM capture ELISA, the P/N ratios for the positive samples ranged from 2- to 10.8-fold. The ODs obtained for the positive samples were similar for the recombinant NiV-N protein-based ELISA and the CDC inactivated-virus-based ELISA system. The samples about which the assays disagreed were mainly weakly positive samples. Differences in assay sensitivity might be one of the reasons causing the divergence.

Evaluation of the recombinant NiV-N protein-based indirect IgG ELISA for swine serum. Sixteen swine serum samples collected from the Veterinary Research Institute, Ipoh, Malaysia, were used for the assessment of the NiV-N-based indirect IgG ELISA for swine serum. For the 16 swine serum samples, the NiV-N-based IgG ELISA and the virus antigen-based CDC IgG ELISA had a concordance rate of 100%, with 6 samples positive and 10 negative for both methods (Table 3).

TABLE 2. Sensitivity and specificity[a] of the NiV-N IgM capture ELISA with reference to the CDC IgM ELISA for human serum

CDC IgM ELISA results	NiV-N IgM ELISA results		Total
	No. positive	No. negative	
No. positive	33	3	36
No. negative	8	89	97
Total	41	92	133

[a] Sensitivity, calculated as (number of true-positive results)/[(number of true-positive results) + (number of false-negative results)] × 100, was 91.7%. Specificity, calculated as (number of true-negative results)/[(number of true-negative results) + (number of false-positive results) × 100, was 91.8%. Concordance, calculated as [(number of samples positive by both methods) + (number of samples negative by both methods)]/(total number of samples) × 100, was 91.7%.

TABLE 3. Sensitivity and specificity[a] of NiV-N-based indirect IgG ELISA with reference to virus antigen-based IgG ELISA for swine serum

Virus antigen-based IgG ELISA results	NiV-N IgG ELISA results		Total
	No. positive	No. negative	
No. positive	6	0	6
No. negative	0	10	10
Total	6	10	16

[a] Sensitivity, calculated as (number of true-positive results)/[(number of true-positive results) + (number of false-negative results)] × 100, was 100%. Specificity, calculated as (number of true-negative results)/[(number of true-negative results) + (number of false-positive results) × 100, was 100%. Concordance, calculated as [(number of samples positive by both methods) + (number of samples negative by both methods)]/(total number of samples) × 100, was 100%.

DISCUSSION

We have successfully cloned the Nipah virus N gene, expressed Nipah virus nucleocapsid protein in *E. coli*, and purified the recombinant protein to near-homogeneity by His tag-based affinity chromatography under native conditions. DNA sequencing of the recombinant plasmid confirmed the insertion of the N gene. The size of the expressed protein was as expected (Fig. 1). The purified NiV-N protein reacted with an antihistidine serum and with human patient serum by Western blotting (Fig. 2). All these results confirmed that the NiV-N protein we obtained was truly the recombinant Nipah virus nucleocapsid protein. The yield of the purified NiV-N protein from 1 liter of bacterial culture was around 1 mg.

The production of recombinant proteins in *E. coli* as capturing antigens for the diagnosis of diseases has been widely used for virological studies (12, 14). Expression of recombinant protein in *E. coli*, however, always produces inactive aggregates (inclusion bodies) in the host cell instead of forming its native, soluble, and biologically active conformation. This is the general rule rather than the exception (13, 19). In this study, by decreasing the culture temperature and reducing the amount of the inducing agent IPTG, we managed to obtain the native soluble form of recombinant NiV-N protein without either using any detergent or performing the arduous work of refolding the denatured protein. The expression and purification procedures described in this study provide a simple and efficient way to obtain pure soluble Nipah virus N protein in large quantities.

Using this recombinant NiV-N protein, we developed an indirect IgG ELISA for human and swine sera and an IgM capture ELISA for human serum, and we compared these with the virus antigen-based ELISAs developed by the CDC. One hundred thirty-three suspected patient sera and 16 swine sera were evaluated using both ELISA systems. For the 133 human sera, as shown in Tables 1 and 2, the NiV-N protein-based ELISA and the CDC ELISA had concordance rates of 98.5% and 91.7% for the indirect IgG ELISA and the IgM capture ELISA, respectively. The sensitivity and specificity of the NiV-N protein-based ELISA systems with regard to the CDC inactivated-virus-based ELISA systems were 98.6% and 98.4% for the indirect IgG ELISA and 91.7% and 91.8% for the IgM capture ELISA, respectively. One sample for the IgG ELISA and three samples for the IgM ELISA were positive by the

CDC ELISA but negative by the NiV-N ELISA. It was speculated that those patients did not develop antibodies to the N protein or that such antibody production was developed at a late phase of infection. In the IgM ELISAs, eight samples were positive by the NiV-N ELISA but negative by the CDC ELISA. These samples are from the patients with infections confirmed by the IgG test. This indicated that the NiV-N-based IgM ELISA is more sensitive than the inactivated-virus-based ELISA. For the 16 swine sera, the two test systems were in 100% concordance (Table 3). Our study is the first report of the use of recombinant NiV-N protein for the diagnosis of human infection. Our data indicate that the Nipah virus nucleocapsid protein is a highly immunogenic protein and a good target for serodiagnosis. Our NiV-N protein-based ELISA systems can be safe and affordable tools for serodiagnosis of Nipah virus infection.

Compared to the inactivated-virus-based-ELISAs, which are currently used by most researchers for diagnosis of Nipah virus infection, the recombinant NiV-N protein-based ELISAs presented in this study offer several distinct advantages. First, the use of infectious virus for antigen production, which requires the highest level of microbiological security and a proper way to inactivate and to monitor the inactivation of the virus, is not required. Hence, ours is a safer method for diagnosis. Second, when antigens obtained from Nipah virus-infected cell extracts are used, it is difficult to standardize the test because of many factors, such as the multiplicity of virus infection, virus strains, cell line, and cell condition, which may generate differences in the relative proportions of the immunoreactive proteins included in the antigen products. Hence the amounts of immunoreactive proteins may be different for different batches of antigen. This feature makes the quantitative interpretation of the test difficult. Therefore, the recombinant protein produced in *E. coli* provides a solution to this problem, allowing easy standardization of antigen production. Third, the recombinant product can be obtained within a relatively short time (within 1 week after cloning), and the expression and purification procedures are simple and easy to perform. The advantages of using a prokaryotic host to produce recombinant N protein can be considerable due to the ease of scale-up and the low costs involved in growing bacteria. This procedure would be especially useful in cases of large-scale epidemiological investigations, as well as in developing countries where high-security laboratories are not available.

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