JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1997, p. 3264–3268 Vol. 35, No. 12
0095-1137/97/$04.00+0

Evaluation of Serological Methods for Diagnosis of Puumala Hantavirus Infection (Nephropathia Epidemica)

KATARINA BRUS SJÖLANDER,[1] FREDRIK ELGH,[2,3] HANNIMARI KALLIO-KOKKO,[4] OLLI VAPALAHTI,[4] MADELEINE HÄGGLUND,[2] VIKTORIA PALMCRANTZ,[2] PER JUTO,[2] ANTTI VAHERI,[4] BO NIKLASSON,[1,3] AND ÅKE LUNDKVIST[1,5]*

Swedish Institute for Infectious Disease Control, S-105 21 Stockholm,[1] Department of Virology, University of Umeå, S-901 85 Umeå,[2] Department of Microbiology, Division of NBC Defence, Defence Research Establishment, S-901 82 Umeå,[3] and Microbiology and Tumour Biology Centre, Karolinska Institute, S-171 77 Stockholm,[5] Sweden, and Department of Virology, Haartman Institute, FIN 00014 University of Helsinki, Finland[4]

Received 4 June 1997/Returned for modification 29 July 1997/Accepted 26 September 1997

Nephropathia epidemica (NE), Puumala (PUU) virus infection, is a febrile disease which is commonly associated with acute renal impairment. To differentiate NE from other acute febrile illnesses, a rapid and reliable serological diagnosis is important, and a number of different protocols have recently been introduced. In the present report we describe a comparative evaluation of six PUU virus immunoglobulin M (IgM) and seven IgG enzyme-linked immunosorbent assay (ELISA) protocols based on native, *Escherichia coli*-expressed, or baculovirus-expressed nucleocapsid protein (N). Neutralization and immunofluorescence assays were included for comparison. Equally high sensitivities and specificities were obtained with three μ-capture-based IgM ELISAs using native, baculovirus-expressed, and *E. coli*-expressed N antigens, respectively, and by an ELISA based on purified *E. coli*-expressed full-length N adsorbed to solid phase. The assays based on truncated amino-terminal N proteins, including a commercially available PUU virus IgM ELISA, all showed lower sensitivities. For detection of PUU virus-specific IgG, ELISAs based on monoclonal antibody-captured native or baculovirus-expressed N antigens showed optimal sensitivities and specificities, while the assays based on *E. coli*-expressed N did not detect all PUU virus IgG-positive serum samples. A commercially available PUU virus IgG ELISA based on *E. coli*-expressed amino-terminal N showed a significantly lower sensitivity than those of all other IgG assays.

Hemorrhagic fever with renal syndrome (HFRS) is a complex of human diseases which occur worldwide and is characterized by fever and renal dysfunction with or without hemorrhagic manifestations. The causative agents of HFRS are hantaviruses belonging to the *Bunyaviridae* family (19, 25, 27). Hantaviruses are enveloped viruses with negative-sense single-stranded RNA genomes. The genome is tripartite and encodes the nucleocapsid protein (S segment); two envelope glycoproteins, G1 and G2 (M segment); and the viral RNA polymerase (L segment) (7, 27). The natural reservoirs of hantaviruses are small rodents or insectivores, and transmission to man is believed to occur via aerosolized excretions (30).

HFRS is a significant public health problem in several countries. Up to 150,000 cases are observed annually in China (2), Korea reports 600 to 1,000 cases per year (14), and several thousands of cases are observed annually in Russia (29). In Sweden and Finland approximately 200 and 1,000 cases, respectively, are diagnosed each year (22, 32). Nephropathia epidemica (NE), the form of HFRS that occurs in Scandinavia, Finland, western Russia, and central Europe is caused by Puumala (PUU) virus (1, 22, 28).

The diagnosis of NE requires serological confirmation. Although PCR has been successfully applied for detection of PUU virus RNA in some patients' samples, the short duration or complete absence of detectable viremia during the acute phase of NE makes PCR unsuitable for diagnosis (10, 26). The nucleocapsid protein (N) of PUU virus has been demonstrated to be the major antigenic target in the early human antibody response, and high levels of N-specific immunoglobulin M (IgM) are produced during the acute phase of NE (3, 17). Thus, N is an essential and sufficient antigen for assays used to diagnose acute PUU virus infections (4, 20, 33). Protocols for detection of hantavirus-specific IgM by enzyme-linked immunosorbent assays (ELISAs) based on native viral antigens have been described previously (11, 23). Due to the hazardous nature of hantaviruses, the handling and preparation of native antigens require biosafety-level-3 facilities. Moreover, hantaviruses, and especially PUU virus, grow slowly and produce low yields of viral antigen in cell culture. Zöller and coworkers (35) reported the expression of recombinant PUU virus N antigen in *Escherichia coli*. Several PUU virus IgM and IgG assays, all utilizing *E. coli*-expressed PUU virus N, have been published during recent years (3, 5, 13, 36, 37). Recently, PUU virus IgM and IgG ELISAs based on recombinant N protein expressed in insect cells have been described (11, 34).

The only commercial assays for HFRS diagnosis available to date are ELISAs for the detection of PUU or Hantaan virus-specific IgM and IgG antibodies, marketed by Progen (Heidelberg, Germany). These kits have been available for several years; however, most laboratories still use in-house diagnostic methods such as immunofluorescence assay (IFA) or various ELISA protocols. The lack of standardized and thoroughly evaluated assays makes comparison of data regarding the prevalence of NE and seroepidemiological data from different geographical regions difficult.

In the present study we evaluated six IgM and seven IgG ELISA protocols based on native, baculovirus-expressed, and

* Corresponding author. Phone: 46-8-7351273. Fax: 46-8-7351424. E-mail: Ake.Lundkvist@smi.ki.se.

E. coli-expressed PUU virus N proteins. Neutralization assay and IFA analyses were included for comparison.

MATERIALS AND METHODS

Sera and patients. Patients' serum samples previously analyzed in the diagnostic routine at the Swedish Institute for Infectious Disease Control (formerly the National Bacteriological Laboratory, Stockholm, Sweden) by a μ-capture IgM ELISA based on native viral antigen, as described below, were used to evaluate the IgM ELISAs. The panel consisted of acute-phase serum samples from 75 serologically confirmed (PUU virus IgM-positive) patients, all with clinical symptoms of NE, and 78 serum samples from patients with clinical suspicion of NE but who were serologically negative.

A panel of 37 PUU virus IgG-positive and 147 IgG-negative serum samples, previously analyzed by a neutralization test (9), from apparently healthy individuals in an area of endemicity for NE in northern Sweden was used for evaluation of the IgG assays.

Antigens. Native PUU virus ELISA antigens were produced as previously described (16, 17). The antigen used for IgM detection consisted of sonicated extracts of infected Vero E6 cells and the IgG ELISA antigen of infected cells treated with detergent buffer. Baculovirus-expressed PUU virus strain Sotkamo full-length N (bac-PUU-N) was produced as previously described (34). Production and affinity purification of *E. coli*-expressed PUU Sotkamo full-length N (rN) and *E. coli*-expressed PUU virus amino-terminal N (rNΔ; amino acids [aa] 1 to 117) have been described previously (3, 6). Briefly, the expressed polyhistidine-containing rN and rNΔ fusion proteins were affinity purified on metal-chelating Sepharose. The rN antigen was concentrated in dialysis tubing on a bed of polyethylene glycol 20,000 and solubilized in 1% sodium dodecyl sulfate (SDS)–1 mM dithiothrenitol. For further purification, the material was separated on an SDS–12% polyacrylamide preparative gel (15). Gel slices corresponding to PUU rN were cut out and eluted in an SDS-polyacrylamide gel electrophoresis (PAGE) running buffer. Suitable working dilutions of the antigens were determined by chessboard titrations. *E. coli*-expressed amino-terminal N (aa 1 to 119) from PUU virus (strain CG-1820) was used in the Progen IgM and IgG ELISAs (26a).

ELISAs. (i) IgM μ-capture ELISAs. PUU virus IgM μ-capture ELISAs based on native N, baculovirus- or *E. coli*-expressed rN and rNΔ antigens were performed as previously described (20, 34). Briefly, microtiter plates were coated with goat anti-human IgM, blocked with bovine serum albumin, and incubated with patients' or control serum samples at a 1:200 dilution. Each antigen was added at optimal concentrations as determined by chessboard titrations, followed by the PUU virus N-specific peroxidase-conjugated bank vole monoclonal antibody (MAb) 1C12 (16). All incubations were done for 1 h at 37°C, except for the 1.5-h incubation with the enzyme-conjugated MAb, and the plates were washed five times between each step. Specific antibody binding was visualized by the addition of a tetramethylbenzidine (TMB) substrate. The reaction was stopped after 12 min of incubation at room temperature, and optical densities at 450 nm (OD_{450}) were determined. Serum samples were tested in duplicate in both antigen-sensitized wells and control wells. To calculate the results, all absorbances were adjusted according to a standard control virus-positive serum: the mean OD value for its duplicate wells was recalculated to 1.000, and the OD values of the remaining wells were adjusted accordingly. Background OD values for the control wells were subtracted from the OD value for wells incubated with antigen. The cutoff value for positive samples was set at an OD value of 0.150.

(ii) IgM ELISAs based on *E. coli*-expressed N bound to solid phase. IgM ELISAs based on *E. coli*-expressed complete or amino-terminal PUU virus N proteins were performed as recently described (3–6). Briefly, microtiter plates were coated with PUU virus rN or rNΔ at 4°C overnight. After being washed once, the plates were blocked for 1 h at room temperature and washed. Sera pretreated with RF-absorbent (Behringwerke AG, Marburg, Germany) and diluted 1:400 were added in duplicate in antigen-sensitized and control wells and incubated for 1 h. After four washes, peroxidase-conjugated goat anti-human IgM $F(ab')^2$ fragments were added. The A_{450} for the specific IgM reaction was determined after 15 min of incubation with TMB.

(iii) IgG ELISAs. PUU virus IgG ELISAs based on MAb-captured native N, baculovirus- or *E. coli*-expressed rN and rNΔ antigens were performed essentially as described previously (17, 34). Briefly, microtiter plates were coated with MAb 1C12 (1 to 10 μg/ml) and incubated overnight at 4°C. After being blocked, antigens were added, and the mixture was incubated, followed by incubation of serum samples (diluted 1:400) in duplicate wells in both antigen-sensitized and control wells. A goat anti-human IgG (γ-chain specific)-alkaline phosphatase conjugate was added. All reagents were added at 100 μl/well except for post-coating, where 150-μl/well volumes were used, and all incubations were done for 1 h at 37°C. PUU virus-specific IgG were detected by the Sigma 104 phosphatase substrate, and OD_{405} values were determined after 30 min of incubation at 37°C. The results were calculated as described above for IgM ELISAs except for the use of a late-convalescent NE serum sample as the standard control and a cutoff value for virus-positive samples set at an OD of 0.100.

IgG ELISAs based on purified and direct-coated *E. coli*-expressed rN or rNΔ (3, 5) were carried out as described above for the rN IgM ELISA except that pretreatment with RF absorbent was excluded and a peroxidase-labeled goat anti-human IgG conjugate was used for detection of specific antibody binding. The IgG ELISA based on rNΔ was evaluated with serum samples diluted both 1:50 and 1:200.

(iv) Progen assays. PUU IgM and IgG ELISAs marketed by Progen were performed according to the manufacturer's instructions.

Neutralization tests (NT). (i) NT-ELISA. The assay was performed as described previously (9). Briefly, sera were serially diluted in tissue culture medium, incubated with PUU virus (strain Sotkamo [31]) for 1 h, and subsequently inoculated onto microtiter plates containing confluent Vero E6 cell monolayers. After incubation for 12 days, the plates were washed and fixed. Rabbit anti-PUU virus antibodies, followed by peroxidase-conjugated goat antibodies to rabbit IgG, were used for detection of PUU virus antigen. After being washed, the viral antigen was visualized by the addition of 2,2′-azinobisdiammonium salt. The A_{405} values were read after 30 min. Specimens were tested in duplicate wells, and the serum dilutions giving a mean reduction of absorbance of more than 80% of that of the negative serum at the corresponding dilution were considered to be neutralizing.

(ii) FRNT. The focus-reduction neutralization test (FRNT) was performed as described previously (24). Briefly, sera were serially diluted and mixed with an equal volume of PUU virus, containing 30 to 70 FFU/100 μl. The mixture was incubated for 1 h and subsequently inoculated into wells of six-well tissue culture plates containing confluent Vero E6 cell monolayer. The wells were overlaid with a mixture of agarose and tissue culture medium and incubated for 12 days. The agarose was removed from the wells, and the cells were fixed. Rabbit anti-PUU virus serum, followed by goat antibodies to rabbit IgG labeled with peroxidase, were added to indicate virus-infected cells. TMB was used as the substrate, and foci were enumerated. An 80% reduction of the number of foci, compared to that of the virus control, was used as the end point for virus neutralization titers.

IFA. Detection of PUU virus IgG by IFA was performed as previously described (22). Vero E6 cells infected with PUU virus (strain Sotkamo) were fixed on spot slides. PUU virus-specific IgG antibodies were detected with fluorescein isothiocyanate-conjugated sheep anti-human IgG.

TABLE 1. Detection of PUU virus IgM

ELISA	No. positive/total no. of virus-positive samples tested (n = 75)	No. negative/total no. of virus-negative samples tested (controls) (n = 78)
IgM μ-capture		
Native N	75/75	78/78
bac-PUU-N (aa 1–433)	75/75	78/78
E. coli rN (aa 1–433)	75/75	78/78
IgM direct-coated		
E. coli rN (aa 1–433)	75/75	78/78
E. coli rNΔ (aa 1–117)	73/75[a]	78/78
Progen IgM *E. coli* rN (aa 1–119)	69/75[b]	78/78

[a] Two serum samples gave negative results (<10 arbitrary units).
[b] Six serum sample gave intermediate (±) results.

RESULTS

Evaluation of PUU virus IgM ELISAs. A total of 153 serum samples from NE patients, or from patients with similar clinical symptoms, all previously examined by μ-capture IgM ELISA based on native viral antigen in the routine diagnosis of patients at the Swedish Institute for Infectious Disease Control, were used for evaluation of six different ELISA protocols (Table 1). The μ-capture ELISAs based on both baculovirus- and *E. coli*-expressed full-length N antigens showed optimal specificity and sensitivity; i.e., all serum samples reacted identically to the results obtained by the μ-capture ELISA based on native antigen.

The IgM ELISA based on direct-coated *E. coli*-expressed complete rN also detected all positive serum samples, while the ELISA based on direct-coated *E. coli*-expressed truncated rNΔ did not detect two of the 75 PUU virus IgM-positive serum samples. The Progen assay (based on *E. coli*-expressed amino-terminal [aa 1 to 119] PUU virus CG-1820 N) indicated six

TABLE 2. Antibody specificity to PUU virus confirmed by FRNT

Patient no.	No. of days after onset of disease	Result for IgM ELISA: Native N (OD_{450})[a]	IgM ELISA: *E. coli* rNΔ (AU)[b]	IgM ELISA: Progen[c]	FRNT[d] (Sotkamo)
1	NA[e]	+ (0.907)	+ (24)	± (1.2)	320
2	8	+ (0.702)	+ (155)	± (1.6)	640
3	NA	+ (0.951)	− (5)	± (1.6)	160
4	3	+ (0.312)	− (9)	± (2.0)	320
5	14	+ (1.016)	+ (49)	± (1.2)	640
6	<7	+ (1.070)	+ (116)	± (1.9)	640

[a] OD_{450} cutoff for positive reaction was >0.150.
[b] AU, arbitrary units. The cutoff for a positive reaction was >15 AU (4).
[c] The calculations were done according to Progen directions ($x < 1$, negative; $1 \leq x \leq 2$, intermediate (±); $x > 2$, positive.
[d] Reciprocal end point titer.
[e] NA, data not available.

TABLE 3. Comparison of IgG assays based on MAb-captured and directly coated rN

ELISA for PUU virus	Cutoff	Sensitivity (%)	Specificity (%)
MAb 1C12 capture			
Gel-purified rN			
Without ECA[a]	0.195	82	100
With ECA	0.026	100	100
Affinity-purified rN			
Without ECA	0.027	100	95
With ECA	0.026	100	100
Direct-coated, affinity-purified rN (with ECA)	0.057	100	80

[a] ECA, *E. coli* extracts.

intermediate samples of the 75 PUU virus IgM-positive serum samples. These six patients all had documented clinical symptoms of acute PUU virus infection (data not shown). However, to confirm the PUU antibody-positivity of the six deviating serum samples (which included the two serum samples found negative by the ELISA based on truncated rNΔ), FRNT was performed. The results showed that all six serum samples contained significant titers of PUU virus-neutralizing antibodies (reciprocal titers ranged between 160 and 640 [Table 2]). All 78 control serum samples were negative by the six different IgM assays.

Finally, we evaluated the suitability of the PUU virus Sotkamo rNΔ antigen in the μ-capture system. No specific antibody reactivity was detected by this format, in spite of the fact that this antigen was almost as sensitive as full-length rN in the ELISA in which the antigen was coated direct to the solid phase.

Detection of PUU virus-specific IgG. Previous studies have revealed the efficient and highly specific detection of PUU virus IgG by assays based on MAb-captured N antigen (17, 18, 21a, 34). Furthermore, several investigators have recently reported problems with unspecific reactions, using *E. coli*-expressed hantavirus N directly coated onto the solid phase (4, 38). As an attempt to improve the sensitivity and specificity of PUU virus IgG assays based on rN, we applied MAb capture of the antigen, purified by two different strategies, and also introduced an *E. coli* antigen for preadsorbtion of unspecific antibodies. These protocols were compared with PUU virus rN-based ELISA developed earlier in which the recombinant antigen was coated directly onto the solid phase (3).

The various ELISA protocols were evaluated with selected serum samples including 22 PUU virus antibody-positive and 20 PUU virus antibody-negative serum samples from healthy individuals living in an area of endemicity for NE (9). Cutoff levels for the different ELISAs were chosen as the means of the results from 39 PUU antibody-negative serum samples (from serologically confirmed acutely ill patients suffering from varicella-zoster virus, measles virus, cytomegalovirus, or influenza A virus infections) + 3 standard deviations.

The results revealed that PUU virus rN antigen that had been coated onto ELISA wells by means of capture by MAb 1C12 resulted in assays with higher sensitivities and fewer background problems than the PUU virus rN ELISA based on direct coating of the antigen (Table 3). The methods were significantly improved by the addition of *E. coli* extracts to the serum dilution buffer. Differences between the protocols with affinity-purified and SDS-PAGE gel-purified antigens were negligible when *E. coli* extracts were used. We chose the SDS-PAGE gel-purified antigen preparation for the final evaluation (see below), since it, at least theoretically, contained the least amount of contaminating *E. coli* remnants.

Evaluation of PUU virus IgG ELISA. Seven IgG ELISA protocols were evaluated with a panel of serum samples previously examined by neutralization test (Table 4). All 36 PUU virus IgG-positive serum samples were found positive by the MAb-antigen-capture ELISAs based on native or baculovirus-expressed antigen. No false-positive reactions were found among the 147 negative serum samples in these two assays. IFA showed a slightly lower sensitivity and detected 34 of the 36 positive serum samples. The ELISA based on MAb-captured *E. coli*-expressed complete rN and truncated rNΔ missed two and one of the positive serum samples, respectively. The ELISA based on direct-coated *E. coli*-expressed rNΔ also failed to detect two of the positive serum samples and showed

TABLE 4. Detection of PUU virus IgG

Assay	No. of samples with result indicated[a]: n = 36 PUU virus antibody-positive samples: +	−	±	n = 147 negative samples (control): −	+	±
NT-ELISA	36			147		
IFA	34	2		147		
IgG MAb-capture ELISA						
Native PUU-N	36			147		
bac-PUU-N (aa 1–433)	36			147		
E. coli rN (aa 1–433)	34	2		147		
E. coli rNΔ (aa 1–117)	35	1		146	1	
IgG direct-coated ELISA						
E. coli rNΔ (aa 1–117) (sera diluted 1:200)	30	6		147		
E. coli rNΔ (aa 1–117) (sera diluted 1:50)	34	2		146	1	
Progen IgG *E. coli* rN (aa 1–119)	22	9	5	145		2

[a] n, total number of serum samples tested.

one false-positive reaction (serum diluted 1:50), while the same assay detected 30 of the 36 positive serum samples assayed at a dilution of 1:200. The Progen PUU virus IgG ELISA detected only 22 of the 36 positive serum samples; 5 samples were scored as intermediates, while 9 were found to be false negatives. The Progen IgG ELISA further detected 2 of the 147 negative control serum samples as intermediates.

DISCUSSION

NE, a form of HFRS commonly encountered in northern Europe, is a febrile disease commonly associated with acute renal impairment. A rapid and reliable serological diagnosis is therefore important in differentiating NE from other acute febrile illnesses in areas of endemicity. One major drawback with the IFA is that a significant proportion of NE patients have high IgG IFA titers in sera during early stages of infection, and seroconversion or a fourfold increase in titer is found in only about half of the patients (3, 22). A variant of this method is the IFA IgG avidity test. This assay can distinguish between specific, low-avidity antibodies representing the acute phase and specific, high-avidity antibodies developed during the late convalescent phase of NE (8). However, 5 to 10% of Swedish NE patients did not develop detectable levels of specific IgG at the time of admission to the hospital (3, 4, 20). Similar results have recently been shown for HFRS patients in Bosnia-Herzegovina, both for PUU and Dobrava virus infections (21).

With its high sensitivity and the capability of processing a large numbers of samples rapidly, ELISA has become the method of choice for the serodiagnosis of hantavirus infections. Results are usually obtained within a few hours and do not suffer from subjective ambiguities, as often encountered with IFA.

Although the amino-terminal region of PUU virus N has been shown to constitute the major antigen target in the human antibody response (5), our recent data have indicated the presence of regions of importance in the humoral response also in the middle and the carboxy-terminal parts of the protein (20, 33). Baculovirus-expressed antigen has, in contrast to *E. coli*-expressed PUU virus N, been found antigenically indistinguishable from the native protein (5, 11, 34).

In an attempt to evaluate and compare some of the different ELISA protocols published during recent years, we used selected panels of PUU virus IgM- and IgG-positive sera. The data from the IgM assays showed that the IgM μ-capture ELISAs based on bac-PUU-N or *E. coli*-expressed full-length N had sensitivity and specificity equivalent to those of the assay based on native virus antigen. The IgM ELISA based on *E. coli*-expressed full-length rN bound to the solid phase also showed optimal sensitivity and specificity. This protocol required serum preincubation with RF absorbent for an efficient detection of specific IgM. The assay based on directly coated rNΔ did not detect PUU virus-specific IgM in 2 of the 75 acute-phase NE serum samples. One likely explanation for the lower sensitivity of this antigen is the presence of antigenic epitopes in other parts of the protein, present only in full-length *E. coli*-expressed rN. Another possibility is that the conformation of rNΔ is not optimal.

The commercial PUU virus IgM ELISA marketed by Progen did not miss any of the virus-positive serum samples, but 6 samples were scored as intermediates. It should be noted that two of the serum samples were obtained more than 1 week after the onset of the disease. Such results require additional serum samples for analysis and thereby eliminates the advantage of a rapid system for IgM detection. The Progen IgM assay is also based on *E. coli*-expressed amino-terminal N (aa 1 to 119). All the IgM assays proved to be highly specific since no false-positive reactions were observed.

When amino-terminal rNΔ (aa 1 to 117) was applied in the IgM μ-capture system, no specific reactivity could be shown. We believe that one explanation for these findings is the nature of the polymers composed of full-length N proteins, as opposed to rNΔ, which is found in solution as free monomers (6a). The polymeric nature of full-length rN and possibly of native N might be one reason for their suitability as antigens in serological assays, in particular for capture assays. Moreover, when the antigen is treated with a strong detergent, discontinuous epitopes may be destroyed.

Unspecific reactions occurred when *E. coli*-expressed antigens were used in IgG assays, probably depending on *E. coli* contaminants remaining in the rN, although extensive purification by affinity chromatography followed by gel purification was done (Table 3). It was further shown that the unspecific absorbances could not be eliminated solely by use of the MAb-captured antigen system. Background reactivity had to be reduced by absorbtion of the serum samples with *E. coli* extracts. Direct comparison of the assays based on directly coated and MAb-captured antigens revealed higher specificities and sensitivities for the latter (Table 3). However, when these systems were compared with the use of the larger panel of serum samples, only minor differences were seen (Table 4), although in line with the first results. The Progen PUU virus IgG assay was shown to be unreliable for identification of long-term immunities, e.g., in seroepidemiological studies (only 22 of the 36 IgG-positive serum sample were detected and 2 of the negative serum samples were interpreted as intermediates). This very low sensitivity could depend on the combination of truncated *E. coli*-expressed antigen (aa 1 to 119) in a direct-coating system. One additional factor may be that the Progen ELISA is based on PUU virus strain CG-1820, which, in contrast to Swedish or central European PUU virus strains, differs from strain Sotkamo in at least one epitope (N-f) situated in aa 1 to 79 of the protein (16).

In comparison to the neutralization test, the two IgG ELISAs based on crude extracts of native and bac-PUU-N antigens were found to be equally efficient for detecting PUU virus-specific antibodies. The assays based on purified *E. coli*-expressed full-length or amino-terminal rN were all found to have slightly lower sensitivities, similar to those for IFA. The high expression level of bac-PUU-N, compared to the low antigen yield and the hazardous nature of native virus culturing, points to the advantage of the baculovirus system. Another advantage of the bac-PUU-N-based systems, in comparison to *E. coli*-expressed rN, is that no purification of the antigen seems to be needed.

Significant serological cross-reactivities are seen among several of the hantaviruses, e.g. within the PUU/Khabarovsk/Tula/Prospect Hill hantavirus group. Although recent data also revealed high levels of cross-reactivities of the human antibodies to PUU and SN hantaviruses, individual variations in the cross-reactivities of the antibodies were seen (6, 12). In addition, assays based on PUU virus antigen do not efficiently detect antibodies to more distantly related hantaviruses, such as Dobrava, Seoul, or Hantaan viruses. Therefore, in areas where serologically distinct hantaviruses pathogenic to man are circulating, e.g., in the former Jugoslavia, tests based on at least two antigens are needed for diagnosis. Several variants ELISA protocols based on recombinant Hantaan virus N have been reported recently (6, 36, 38). Commercial Hantaan IgM and IgG ELISA kits are available from Progen. The efficiency of these different assays awaits evaluation.

In conclusion, our study showed that the specificities and sensitivities of the PUU virus IgM assays based on either native or recombinant full-length N proteins were equal. The IgG assays based on MAb-captured, native, or baculovirus-expressed N showed results identical to those by a neutralization assay for a panel of sera from healthy individuals from northern Sweden. All assays based on truncated *E. coli*-expressed N were found to be less sensitive and/or specific.

ACKNOWLEDGMENTS

We thank Progen GmbH, Heidelberg, Germany, for the kind gift of PUU virus ELISA kits.

This work was supported by the Swedish Medical Research Council, grants 11230 and 12177 (Stockholm) and 10382 (Umeå); by grants from the Kempe Foundation, the Joint Committee of the Northern Swedish Health Care region, and Förenade Liv Mutual Group Life Insurance Company, Stockholm, Sweden; and by the Medical Research Council of Academy of Finland and Sigrid Jusélués Foundation, Helsinki, Finland.

REFERENCES

1. **Brummer-Korvenkontio, M., A. Vaheri, T. Hovi, C.-H. von Bonsdorff, J. Vuorimies, T. Manni, K. Penttinen, N. Oker-Blom, and J. Lähdevirta.** 1980. Nephropathia epidemica. Detection of antigen in bank voles and serologic diagnosis of human infection. J. Infect. Dis. **141:**131–134.
2. **Chen, H. X., F. X. Qiu, B. J. Dong, S. Z. Ji, Y. Wang, H. M. Wang, G. F. Zuo, X. X. Tao, and S. Y. Gao.** 1986. Epidemiological studies on hemorrhagic fever with renal syndrome in China. J. Infect. Dis. **154:**349–398.
3. **Elgh, F., G. Wadell, and P. Juto.** 1995. Comparison of the kinetics of Puumala virus specific IgM and IgG antibody responses in nephropathia epidemica as measured by a recombinant antigen-based enzyme-linked immunosorbent assay and an immunofluorescence test. J. Med. Virol. **45:**146–150.
4. **Elgh, F., M. Linderholm, G. Wadell, and P. Juto.** 1996. The clinical usefulness of a Puumala virus recombinant nucleocapsid protein based enzyme-linked immunosorbent assay in the diagnosis of nephropathia epidemica as compared to an immunofluorescence assay. Clin. Diagn. Virol. **6:**17–26.
5. **Elgh, F., Å. Lundkvist, O. A. Alexeyev, G. Wadell, and P. Juto.** 1996. A major antigenic domain of the human humoral response to the Puumala virus nucleocapsid protein is located at the amino-terminus. J. Virol. Methods **59:**161–172.
6. **Elgh, F., Å., Lundkvist, O. A. Alexeyev, T. Avsic-Zupanc, B. Hjelle, H. W. Lee, C. Schmaljohn, R. Vainionpää, D. Wiger, G. Wadell, and P. Juto.** 1997. Antibody responses in hantavirus diseases detected by an enzyme-linked immunosorbent assay based on recombinant nucleocapsid proteins of five viral serotypes. J. Clin. Microbiol. **35:**1122–1130.

6a. **Elgh, F., et al.** Unpublished observation.

7. **Elliott, R. M.** 1990. Molecular biology of the Bunyaviridae. J. Gen. Virol. **71:**501–522.
8. **Hedman, K., A. Vaheri, and M. Brummer-Korvenkontio.** 1991. Rapid diagnosis of hantavirus disease with an IgG-avidity assay. Lancet **338:**1353–1356.
9. **Hörling, J., Å. Lundkvist, J. W. Huggins, and B. Niklasson.** 1992. Antibodies to Puumala virus in humans determined by neutralization test. J. Virol. Methods **39:**139–147.
10. **Hörling, J., Å. Lundkvist, K. Persson, M. Mullaart, A. Dekonenko, T. Tkachenko, and B. Niklasson.** 1995. Detection and subsequent sequencing of Puumala virus from human specimens by polymerase chain reaction. J. Clin. Microbiol. **33:**277–282.
11. **Ivanov, A., O. Vapalahti, H. Lankinen, E. Tkachenko, A. Vaheri, B. Niklasson, and Å. Lundkvist.** 1996. Biotin-labeled antigen: a novel approach for detection of Puumala virus-specific IgM. J. Virol. Methods **62:**87–92.
12. **Jenison, S., T. Yamada, C. Morris, B. Anderson, N. Torrez-Martinez, N. Keller, and B. Hjelle.** 1994. Characterization of human antibody responses to four corners hantavirus infections among patients with hantavirus pulmonary syndrome. J. Virol. **68:**3000–3006.
13. **Kallio-Kokko, H., O. Vapalahti, K. Hedman, M. Brummer-Korvenkontio, and A. Vaheri.** 1993. Puumala virus antibody and immunoglobulin G avidity assays based on a recombinant nucleocapsid antigen. J. Clin. Microbiol. **31:**677–680.
14. **Kim, G. R., Y. T. Lee, and C. H. Park.** 1994. A new natural reservoir of hantavirus: isolation of hantaviruses from lung tissues of bats. Arch. Virol. **134:**85–95.
15. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680–685.
16. **Lundkvist, Å., A. Fatouros, and B. Niklasson.** 1991. Antigenic variation of European haemorrhagic fever with renal syndrome virus strains characterized using bank vole monoclonal antibodies. J. Gen. Virol. **72:**2097–2103.
17. **Lundkvist, Å., J. Hörling, and B. Niklasson.** 1993. The humoral response to Puumala virus infection (nephropathia epidemica) investigated by viral protein specific immunoassays. Arch. Virol. **130:**121–130.
18. **Lundkvist, Å., S. Björsten, and B. Niklasson.** 1993. Immunoglobulin G subclass responses against the structural components of Puumala virus. J. Clin. Microbiol. **31:**368–372.
19. **Lundkvist, Å., and B. Niklasson.** 1994. Hemorrhagic fever with renal syndrome and other hantavirus infections. Rev. Med. Virol. **4:**177–184.
20. **Lundkvist, Å., S. Björsten, B. Niklasson, and N. Ahlborg.** 1995. Mapping of B-cell determinants in the nucleocapsid protein of Puumala virus: definition of epitopes specific for acute IgG recognition in humans. Clin. Diagn. Lab. Immunol. **2:**82–86.
21. **Lundkvist, Å., M. Hukic, J. Hörling, M. Gilljam, S. Nichol, and B. Niklasson.** 1997. Puumala and Dobrava viruses cause haemorrhagic fever with renal syndrome in Bosnia-Herzegovina: evidence of highly cross-neutralizing antibody responses in early patient sera. J. Med. Virol. **53:**51–59.

21a. **Lundkvist, Å., et al.** Unpublished data.

22. **Niklasson, B., and J. W. LeDuc.** 1987. Epidemiology of nephropathia epidemica in Sweden. J. Infect. Dis. **155:**269–276.
23. **Niklasson, B., and T. Kjelsson.** 1988. Detection of nephropathia epidemica (Puumala virus)-specific immunoglobulin M by enzyme-linked immunosorbent assay. J. Clin. Microbiol. **26:**1519–1523.
24. **Niklasson, B., M. Jonsson, Å. Lundkvist, J. Hörling, and E. Tkachenko.** 1991. Comparison of European isolates of viruses causing hemorrhagic fever with renal syndrome by a neutralization test. Am. J. Trop. Med. Hyg. **45:**660–665.
25. **Plyusnin, A., O. Vapalahti, and A. Vaheri.** 1996. Hantaviruses: genome structure, expression and evolution. J. Gen. Virol. **77:**2677–2687.
26. **Plyusnin, A., J. Hörling, M. Kanerva, J. Mustonen, Y. Cheng, J. Partanen, O. Vapalahti, S. K. J. Kukkonen, J. Niemimaa, H. Henttonen, B. Niklasson, Å. Lundkvist, and A. Vaheri.** 1997. Puumala hantavirus genome in patients with nephropathia epidemica: correlation of PCR positivity with HLA haplotype and link to viral sequences in local rodents. J. Clin. Microbiol. **35:**1090–1096.

26a. **Progen.** Personal communication.

27. **Schmaljohn, C. S., S. E. Hasty, J. M. Dalrymple, J. W. LeDuc, H. W. Lee, C.-H. von Bonsdorff, M. Brummer-Korvenkontio, A. Vaheri, T. F. Tsai, H. L. Regnery, D. Goldgaber, and P. W. Lee.** 1985. Antigenic and genetic properties of viruses linked to hemorrhagic fever with renal syndrome. Science **227:**1041–1044.
28. **Sheshberadaran, H., B. Niklasson, and E. A. Tkachenko.** 1988. Antigenic relationship between hantaviruses analysed by immunoprecipitation. J. Gen. Virol. **69:**2645–2651.
29. **Tkachenko, E. A., M. A. Donets, G. V. Rezapkin, T. K. Dzagurova, A. P. Ivanov, E. V. Leshchinskaya, E. Reshetnikov, S. G. Drozdov, A. R. Slonova, and G. P. Somov.** 1982. Serotypes of HFRS (haemorrhagic fever with renal syndrome) virus in east European and far eastern U. S. S. R. Lancet **i:**863.
30. **Tsai, T. F.** 1987. Hemorrhagic fever with renal syndrome: mode of transmission to humans. Lab. Anim. Sci. **3:**428–430.
31. **Vapalahti, O., H. Kallio-Kokko, E.-M. Salonen, M. Brummer-Korvenkontio, and A. Vaheri.** 1992. Cloning and sequencing of Puumala virus Sotkamo strain S and M RNA segments: evidence for strain variation in hantaviruses and expression of the nucleocapsid protein. J. Gen. Virol. **73:**829–838.
32. **Vapalahti, O., H. Henttonen, and A. Vaheri.** 1995. Puumala virus infections in Finland. Eurosurveillance, September 1995.
33. **Vapalahti, O., H. Kallio-Kokko, A. Närvänen, I. Julkunen, Å. Lundkvist, A. Plyusnin, H. Lehväslaiho, M. Brummer-Korvenkontio, A. Vaheri, and H. Lankinen.** 1995b. Human B-cell epitopes of Puumala virus nucleocapsid protein, the major antigen in early serological response. J. Med. Virol. **46:**293–303.
34. **Vapalahti, O., Å., Lundkvist, H. Kallio-Kokko, K. Paukku, I. Julkunen, H. Lankinen, and A. Vaheri.** 1996. Antigenic properties and diagnostic potential of Puumala virus nucleocapsid protein expressed in insect cells. J. Clin. Microbiol. **34:**119–125.
35. **Zöller, L., S. Yang, and M. Zeyer.** 1991. Rapid diagnosis of haemorrhagic fever with renal syndrome due to hantavirus. Lancet **338:**183.
36. **Zöller, L., S. Yang, P. Gött, E. K. Bautz, and G. Darai.** 1993. Use of recombinant nucleocapsid proteins of the Hantaan and nephropathia epidemica serotypes of hantaviruses as immunodiagnostic antigens. J. Med. Virol. **39:**200–207.
37. **Zöller, L. G., S. Yang, P. Gött, E. K. F. Bautz, and G. Darai.** 1993. A novel μ-capture enzyme-linked immunosorbent assay based on recombinant proteins for sensitive and specific diagnosis of hemorrhagic fever with renal syndrome. J. Clin. Microbiol. **31:**1194–1199.
38. **Zöller, L., M. Faulde, H. Meisel, B. Ruh, P. Kimming, U. Schelling, M. Zeier, P. Kulzer, C. Becker, M. Roggendorf, E. K. F. Bautz, D. H. Kruger, and G. Darai.** 1995. Seroprevalence of hantavirus antibodies in Germany as determined by a new recombinant enzyme immunoassay. Eur. J. Clin. Microbiol. Infect. Dis. **14:**305–313.