

Quality control measures for the serological diagnosis of hantavirus infections

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Abstract

Background: With society's rapidly increasing mobility, patients infected with severe viral infections can become seriously ill at any place in Europe and elsewhere. Improving the diagnostics of these infections is the most important step in detecting the pathogens and dealing with them, and for this purpose, quality control measures are essential tools. **Objectives:** To assess the diagnostic reality for rare hantavirus infections in Europe by (1) running a pre-evaluation panel (four samples, sent out in 1999) to optimise sample preparation and shipping procedure and afterwards (2) starting an External Quality Assurance (EQA) program (20 samples, sent out in 2001). **Study design:** All samples sent out had to be tested for the presence of specific IgG and IgM antibodies against hantavirus. For the pre-evaluation panel, four samples were distributed (two samples IgG+/IgM-, one sample IgG-borderline/IgM-, one sample IgG-/IgM-), for the EQA 20 samples (six samples IgG+/IgM+, eight samples IgG+/IgM-, one sample IgG-borderline/IgM-, five samples IgG-/IgM-). Thirteen laboratories took part in the pre-evaluation panel, 18 laboratories participated in the first EQA run. **Results:** For the pre-evaluation panel, the participants reported correct results for 64% of the IgG-positive samples (85% excluding borderline-positive sample), and 92% for the IgG-negative sample. IgM testing was correctly negative in all laboratories. For the EQA, the participants reported correct results for 76% of the IgG-positive samples, and 97% correct results for the IgG-negative samples. For the IgM-positive samples, 53% correct results were reported, and 98% correct results for the IgM-negative samples. **Conclusions:** The results presented here prove the importance of quality measures also for viruses only rarely suspected, like hantavirus, and they clearly demonstrate the need for improvement of the existing test systems.

Abbreviations: EIA, enzyme immunoassay; ENIVD, European network for the diagnostics of 'imported' viral diseases; EQA, external quality assurance scheme; HFRS, haemorrhagic fever with renal syndrome; IB, immunoblot; IFA, immunofluorescence assay; PEP, pre-evaluation panel.

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1. Introduction

Considerable attention has recently been directed to emerging and re-emerging infections in national and international discussions, and we all became aware that severe infections could be imported to and/or spread over Europe in a very short time. The improvement of diagnostics of these viral infections is the most important step in detecting the pathogens and dealing with them.

For a number of viral pathogens, e.g. HIV, herpes simplex, cytomegalovirus, enteroviruses, several proficiency panels for the external quality assurance (EQA) of viral diagnostics have been designed (Schweiger et al., 1997; Muir et al., 1999). These EQA panels are offered all over Europe by commercial organisations, e.g. the UK NEQAS (Lewis, 1995; see also <http://www.ukneqas.org.uk>) or INSTAND e.V. (Wood, 1991; see also <http://www.instand-ev.de>), or by the initiative of scientific societies, e.g. the European Union Quality Control Concerted Action raised by the ESCV (see <http://www.qcca.org.uk>). They are aiming at the improvement of the reliability and quality of the diagnostic output. However, there is still a number of rare (i.e. non-profit for diagnostic laboratories) but nevertheless severe viral pathogens beyond these efforts, e.g. hantaviruses.

Hantaviruses, members of the *Bunyaviridae* family, are endemic throughout the world and naturally hosted by rodents. The vast majority of human hantavirus infections are asymptomatic. Symptomatic infections in Europe are summarised as ‘haemorrhagic fever with renal syndrome’ (HFRS) and go along with fever (3–4 days, > 38 °C), headache, flank and abdominal pain. Moreover, renal dysfunction can lead to acute renal failure. HFRS is not common in most of Europe; the local incidence varies from 100 cases per year in Eastern and Southern Europe up to 1000 cases per year only in Finland. The diagnosis is often made with in-house or commercial tests undergoing internal evaluation (Brus Sjölander et

al., 1997), but not subjected to external quality control.

To assess the quality of the hantavirus diagnostics for Europe, the European Network for the Diagnostics of ‘Imported’ Viral Diseases (ENIVD) (<http://www.enivd.de>) distributed two sample sets to be tested for the presence of antibodies directed against hantaviruses, a Pre-evaluation Panel with four samples in 1999 and an EQA serum panel with 20 samples.

2. Material and methods

2.1. Hantavirus diagnostics pre-evaluation panel (PEP)

2.1.1. Serum samples

Two human serum samples (TM, AP) were obtained from A. Vaheri, Helsinki. Sample TM was positive for IgG-antibodies against *Puumala* hantavirus (immunofluorescence titre 3200) and negative for IgM-antibodies; the other serum (AP) was negative for both hantavirus-specific IgG and IgM antibodies. A total number of four samples was sent out. Three serial dilutions of serum TM in PBS (#1 1:10, #2 1:100, #3 1:1,000), and undiluted serum AP was sent out as sample #4.

2.1.2. Distribution

The samples were refrigerated to 4 °C and sent out in aliquots of 1 ml by express mail. Before shipping, the serum panel was evaluated in duplicate with immunoblot (recomBlot Bunyavirus, Mikrogen, Martinsried) according to manufacturer’s instruction. The samples were serologically examined for the presence of IgG and IgM antibodies against hantaviruses by the participating laboratories.

2.1.3. Participants

The following 13 laboratories took part in the hantavirus PEP: Anna Papa, Thessaloniki, Greece;

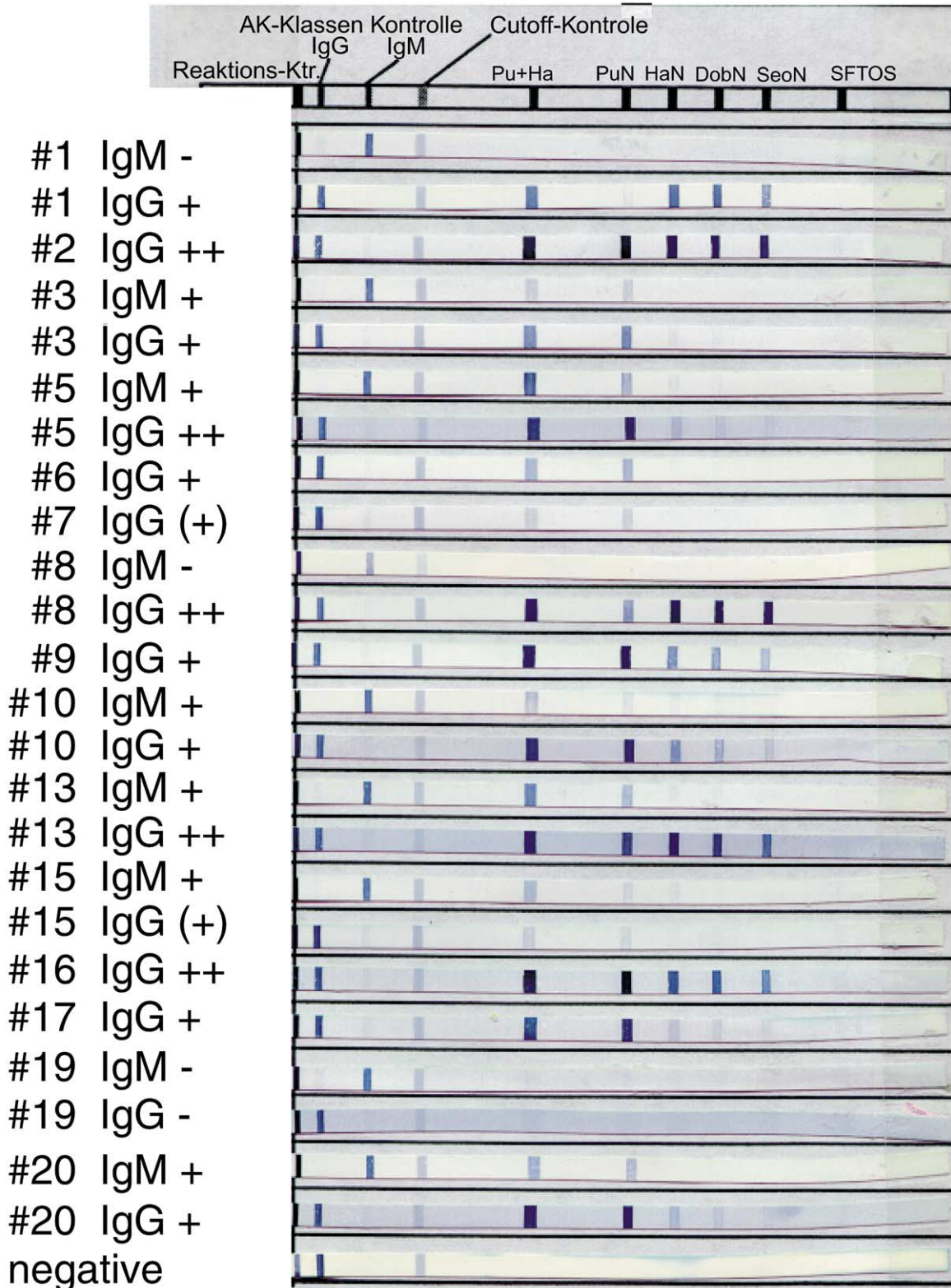


Fig. 1. Immunoblot strips of the IgM and IgG positive samples. Negative samples except one are not shown.

Table 1
Samples for the first hantavirus EQA

Number	Sample type	Dilution ^a	IgM ^b	IgG ^b	Origin	Hantavirus strain	Serum status ^c
#1	Positive serum	1:10	–	+	Kosovo	Dobrava	Convalescent
#2	Positive serum	1:2	–	++	Sweden	Puumala	Convalescent
#3	Positive serum ^f	1:25	+	++	Germany	Non-serotyped	Acute
#4	Negative serum	1:2	–	–	Germany	–	Control
#5	Positive serum	1:2	+	++	Sweden	Puumala	Acute
#6	Positive serum	1:100	–	+	Finland	Puumala	Convalescent
#7	Positive serum	1:1000	–	+	Finland	Puumala	Convalescent
#8	Positive serum	Undiluted	–	+	Kosovo	Dobrava	Convalescent
#9	Positive serum	1:2	–	++	Sweden	Puumala	Convalescent
#10	Positive serum	1:2	+	+	Sweden	Puumala	Acute
#11	Borderline serum ^{f,d}	1:10	–	–	Germany	Non-serotyped	Borderline
#12	Negative plasma	Undiluted	–	–	Germany	–	Control
#13	Positive serum	1:2	+	++	Sweden	Puumala	Acute
#14	Negative serum ^f	1:10	–	–	Germany	–	Control
#15	Positive serum ^f	1:16	+	+	Germany	Non-serotyped	Acute
#16	Positive serum	1:2	–	++	Sweden	Puumala	Convalescent
#17	Positive serum	1:10	–	+	Finland	Puumala	Convalescent
#18	Negative serum	1:2	–	–	Germany	–	Control
#19	Negative serum ^{f,e}	Undiluted	–	–	Germany	–	Control
#20	Positive serum	1:2	+	+	Sweden	Puumala	Acute

–, Negative, +, positive, ++, strong positive.

^a Diluent plasma (sample 12).

^b Tested in duplicate by immunoblot and/or by pre-check laboratories.

^c As indicated by donor laboratories.

^d Borderline positive in Hanta IFA.

^e Multireactive (highly unspecific IgM reaction).

^f Pooled sera.

Antti Vaheri, Helsinki, Finland; Christian Vandenvelde Brussels, Belgium; Filipe Armindo, Águas de Moura, Portugal; Jan Groen, Rotterdam, Netherlands; Stephan Aberle, Wien, Austria; Vincent Deubel, Paris, France; Herbert Schmitz, Hamburg, Germany; Tatjana Avsic Zupanc, Ljubljana, Slovenia; Juan Garcia Costa, Orense, Spain; Helga Meisel, Berlin, Germany; Marjan van Esbroeck, Antwerpen, Belgium; Werner Slenczka, Marburg, Germany.

2.2. First hantavirus diagnostics external quality assurance (EQA)

2.2.1. Serum samples

The first EQA panel, consisted of 20 human serum samples kindly provided by members of the ENIVD network (Å. Lundkvist, Stockholm, A. Vaheri, Helsinki, M. Hukic, Tuzla, P. Emmerich, Hamburg). Six samples were positive for IgG and

IgM antibodies against hantaviruses, eight samples were IgG positive and IgM negative, and one sample was borderline positive for hantavirus specific IgG antibodies and IgM negative. Five samples were negative for both hantavirus-specific IgG and IgM antibodies analysed by IFA and IB. One multireactive (highly unspecific IgM reaction) serum pool from persons with rheumatic disease history was kindly provided by EUROIMMUN, Lübeck, Germany. Serum samples of low quantity but with high antibody titre were diluted with human plasma, negative for hantavirus-specific IgG and IgM antibodies (for detailed information on the serum panel see Table 1, sample 12). For evaluation of test sensitivity one highly positive serum was provided at different dilutions (#17 1:10, #6 1:100, #7 1:1000).

Table 2
Tests applied in the first hantavirus EQA

	Test system	Hantavirus antigens	Manufacturer
Lab A	EIA	Hantaan, Puumala	Progen, Heidelberg
Lab B	IFA (only IgG)	Hantaan, Puumala, Dobrava	In house
	EIA	Hantaan, Puumala, Dobrava	In house
Lab C	EIA	Hantaan, Puumala	Progen, Heidelberg
Lab D	EIA	Hantaan	MRL, NC, USA
Lab E	EIA, IFA, Blot	–	In house
	EIA, IFA	Hantaan, Puumala	Progen, Heidelberg
	Blot	Hantaan, Puumala, Dobrava	Microgen, Martinsried
Lab F	IFA (only IgG)	Hantaan, Puumala	In house
	EIA	Hantaan, Puumala	In house
Lab G	IFA (only IgG)	–	In house
	EIA	Hantaan, Puumala	Progen, Heidelberg
Lab H	IFA	Hantaan, Puumala	Progen, Heidelberg
Lab I	EIA	Hantaan	MRL, NC, USA
	Blot	Hantaan, Puumala, Dobrava	Microgen, Martinsried
Lab J	IFA	Hantaan, Puumala	In house
Lab K	IFA	Hantaan, Puumala	In house
Lab L	IFA	Hantaan, Puumala	In house
Lab M	EIA, IFA	Hantaan, Puumala	In house
Lab N	EIA	Puumala, Dobrava	In house
Lab O	μ -capture EIA (only IgM)	Puumala, Dobrava	In house
	IFA (only IgG)	Puumala, Dobrava	In house
Lab P	EIA (only IgM)	Puumala	In house
	EIA (only IgG)	Hantaan, Puumala	In house
Lab Q	EIA, IFA	Hantaan, Puumala	Progen, Heidelberg
Lab R	IFA	–	In house

2.2.2. Distribution

The 20 samples were aliquoted at 100 μ l in 0.5 ml plastic tubes (Sarstedt, Germany) and frozen at -70 °C before freeze-dried for 12 h (Christ, Alpha I-5, Hanau, Germany). Before shipping, the serum panel was evaluated in duplicate by immunoblotting (recomBlot Bunyavirus, Mikrogen, Martinsried). In this pre-check two sets of samples were evaluated; one set freeze-dried and one set not freeze-dried aliquots.

All samples were sent out by regular mail and arrived within 1 week after sending according to the information for date of arrival as requested from the participants. It was recommended to suspend the samples with 100 μ l distilled water and to centrifuged them for 5 min to remove any aggregates before testing. The samples were to be serologically examined for the presence of IgG and IgM antibodies against hantaviruses by the participating laboratories. There was no obligation

concerning which test procedure should be used. Table 2 gives further details on the tests applied by the participating laboratories.

2.2.3. Participants

The following 18 laboratories took part in the hantavirus EQA: Fernando de Ory, Majadahonda, Spain; Tatjana Avsic-Zupanc, Ljubljana, Slovenia; Christian Vandenvelde, Brussels, Belgium; Detlev Schultze, St. Gallen, Switzerland; Helga Meisel, Berlin, Germany; Bernadette Murgue, Paris, France; Anna Papa, Thessaloniki, Greece; Werner Slenczka, Marburg, Germany; Stephan Aberle, Wien, Austria; Petra Emmerich, Hamburg, Germany; Marjan van Esbroeck, Antwerpen, Belgium; Maria Joao Alves, Águas de Moura, Portugal; Felicity Burt, Johannesburg, Rep. South Africa; Åke Lundkvist, Stockholm, Sweden; Olli Vapalahti, Helsinki, Finland; Heinz Feld-

Table 3
Hantavirus EQA results from IgM-testing

Sample	#5	#13	#10	#20	#15	#3	#2	#6	#9	#19	#1	#4	#7	#8	#11	#12	#14	#16	#17	#18
Status*	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lab A	+ ^P	b ^P	–	b ^P	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lab B	+ ^P	+ ^P	+ ^P	+ ^P	+ ^P	+ ^P	+ ^P	–	+ ^P	–	–	–	–	–	–	–	–	–	–	–
Lab C	+ ^P	+ ^P	b ^P	+ ^P	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lab D	+	+	b	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lab E	+ ^P	+ ^P	+ ^P	b ^P	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lab F	+ ^P	+ ^P	+ ^P	+ ^P	+ ^P	+ ^P	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lab G	b	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lab H	–	–	+ ^P	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lab I	+ ^P	+ ^H	+ ^H	+ ^P	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lab J	+ ^P	+ ^P	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lab K	+ ^P	+ ^P	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lab L	–	+	–	–	–	–	–	–	–	b	–	–	–	–	–	–	–	–	–	–
Lab M	+	–	–	–	–	–	n.t.	–	–	–	–	–	–	–	–	–	–	–	–	–
Lab N	+ ^P	+ ^P	+ ^P	+ ^P	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lab O	+ ^P	+ ^P	+ ^P	+ ^P	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lab P	+ ^P	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lab Q	+ ^P	+ ^P	+ ^P	+ ^P	+ ^P	–	–	+ ^P	–	–	–	–	–	–	–	–	–	–	–	–
Lab R	+	–	+	n.t.	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
Overall concurrent [#]	16	13	11	9	5	3	15	17	17	17	18	18	18	18	18	18	18	18	18	18
	89%	72%	61%	53%	28%	17%	88%	94%	94%	94%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%

–, Negative; +, positive; b, borderline positive; n.t., not tested; *, serostatus (for details Table 1); #, including borderline positive results; P, serotyping result: Puumala; H, serotyping result: Hantaan; D, serotyping result: Dobrava; u, highly unspecific reaction.

Table 4
Hantavirus EQA results from IgG-testing

Sample	#2	#5	#8	#10	#13	#9	#16	#20	#17	#3	#1	#15	#6	#7	#12	#18	#19	#4	#11	#14
Status*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–
Lab A	+ ^{P,H}	+ ^P	+ ^H	+ ^{P,H}	+ ^{P,H}	+ ^P	+ ^P	+ ^P	–	+ ^P	–	–	–	–	–	–	–	–	–	–
Lab B	+ ^P	+ ^P	+ ^D	+ ^P	+ ^P	+ ^P	+ ^P	+ ^P	+ ^P	+ ^P	–	+ ^P	–	–	–	–	–	–	–	–
Lab C	+ ^{P,H}	+ ^P	+ ^H	+ ^{P,H}	+ ^{P,H}	+ ^P	+ ^P	+ ^P	–	+ ^P	–	–	–	–	–	–	–	–	–	–
Lab D	+	+	+	+	+	+	+	+	–	–	–	+	–	–	–	–	–	–	–	–
Lab E	+ ^P	+ ^P	+ ^{H,D}	+ ^P	+ ^P	+ ^P	+ ^P	+ ^P	+ ^P	+ ^P	+ ^{H,D}	–	–	–	–	–	–	–	–	–
Lab F	+ ^H	+ ^P	+ ^H	+ ^P	+ ^H	+ ^P	+ ^P	+ ^P	+ ^P	+ ^P	+ ^H	–	+ ^P	–	–	–	–	–	–	–
Lab G	+ ^H	+	+ ^H	+ ^H	+ ^H	+	+	+	b	–	b ^P	–	–	–	–	+ ^H	+ ^u	–	–	–
Lab H	+ ^D	+ ^P	+ ^D	+ ^P	b ^P	+ ^D	+ ^P	–	+ ^D	+ ^P	+ ^{P,H}	+ ^P	+ ^P	+ ^P	–	–	+ ^u	–	–	–
Lab I	+ ^P	+ ^P	+ ^H	+ ^H	+ ^H	+ ^P	+ ^P	+ ^P	+ ^P	–	+ ^H	b	–	–	–	–	–	–	–	–
Lab J	+ ^P	+ ^P	+	+ ^P	+ ^P	+ ^P	+	+ ^P	+ ^P	+ ^P	+	+ ^P	–	–	–	–	–	–	–	–
Lab K	+ ^P	+ ^P	+ ^H	+ ^P	+ ^P	+ ^P	+	+ ^P	+ ^P	+ ^P	–	+ ^P	+ ^P	–	–	–	+ ^u	–	–	–
Lab L	+	+	+	+	b	+	+	+	+	b	+	–	–	–	–	–	–	–	–	–
Lab M	n.t.	b	+	b	b	+	+	b	+	–	+	–	–	–	–	–	–	–	–	–
Lab N	+ ^P	+ ^P	+ ^D	+ ^P	+ ^P	+ ^P	+ ^P	+ ^P	+ ^P	–	–	–	–	–	–	–	–	–	–	–
Lab O	+ ^P	+ ^P	+ ^{H,D}	+ ^P	+ ^P	+ ^P	+ ^P	+ ^P	+ ^P	+	+ ^{H,D}	+	+ ^P	–	–	–	–	–	–	–
Lab P	+ ^P	+ ^P	+ ^H	+ ^P	+ ^P	+ ^P	+ ^P	b ^P	+ ^P	–	–	–	–	–	–	–	–	–	–	–
Lab Q	+ ^H	+ ^P	+ ^P	+ ^P	+ ^P	+ ^P	+ ^P	+ ^P	b ^P	b	+ ^H	+ ^P	–	–	–	–	–	–	–	–
Lab R	+	+	+	+	+	–	–	+	+	–	–	–	+	–	+	–	–	–	–	–
Overall concurrent [#]	17	18	18	18	18	17	17	17	15	11	10	8	5	1	17	17	17	18	18	18
	100%	100%	100%	100%	100%	94%	94%	94%	83%	61%	56%	44%	28%	6%	94%	94%	94%	100%	100%	100%

–, Negative; +, positive; b, borderline positive; n.t., not tested. *, Serostatus (for details Table 1); #, including borderline positive results; P, serotyping result: Puumala; H, serotyping result: Hantaan; D, serotyping result: Dobrava; u, highly unspecific reaction.

Table 5
Comparison of different test systems used in the hantavirus EQA

Test system	IgM testing concurrent results ^a (%)	IgG testing concurrent results ^a (%)
In house	83	80
Commercial	87	81
IB	87	90
EIA	87	77
IFA	80	85

IB, immunoblot; EIA, enzyme immunoassay; IFA, immunofluorescence assay.

^a Concurrent results of positive and negative results.

mann, Winnipeg, Canada; Jan Groen, Rotterdam, Netherlands; Juan Garcia Costa, Orense, Spain.

After the return of the results of all participants all laboratories received the overview of the test results from all participating laboratories in anonymous form with only their own laboratory indicated.

3. Results

3.1. Hantavirus diagnostics PEP

For the hantavirus PEP the participants reported 64% correct results for the three IgG-positive samples (85% excluding borderline positive sample #3) and 92% correct results were reported for the IgG-negative sample #4 (results not shown). IgM-testing was correctly reported negative for samples #1–#4 by all participating laboratories (100%).

3.2. Hantavirus diagnostics EQA

According to the different pre-checks of the samples we could not find any significant differences in the diagnostic results between the freeze-dried and the native material (results not shown). The human plasma (#12) used for dilution of the specimens do not show any unspecific background reaction influencing the diagnostic results. Table 1 gives an overview on the samples used for the EQA and shows the results obtained in the different pre-

check test systems. Fig. 1 shows the immunoblot of the positive samples. It can be clearly seen that the serum used at different dilutions (#17 1:10, #6 1:100, #7 1:1000) shows a gradient of staining for the N-protein. The results from the hantavirus EQA are presented in detail in Table 3 (IgM testing) and Table 4 (IgG testing). Overall, the participants reported correct results for 76% of the IgG-positive samples, and 97% correct results for the IgG-negative samples. For the IgM-positive samples 53% correct results were reported, and 98% correct results for the IgM-negative samples. The indication of the hantavirus strains given by the laboratory mostly refer to specification of the respectively diagnostic tests used.

The performance of in-house tests used in the EQA was equal to that of commercial test systems for both IgM (in house: 83%, commercial: 87%), and IgG detection (in house: 80%, commercial: 81%). The different test systems immunoblot, EIA and IFA also showed a similar performance (IgM testing IB: 87%, EIA: 87%, IFA: 80%; IgG testing IB: 90%, EIA: 77%, IFA: 85%), however, immunoblot analysis seemed to be slightly more sensitive than EIA or IFA (see also Table 5).

4. Discussion

In this study the results from two quality checks for the serodiagnosis of hantavirus infections in Europe are presented. While the first run (PEP) mainly aimed at optimising sample preparation and shipping procedures, the data from the second run (EQA) provided a good overview of hantavirus diagnostics in Europe.

Looking at the overall results, the trend from the pre-evaluation panel in 1999 has been proven by the EQA scheme in 2001: while the specificity of the hantavirus test systems within the participating laboratories seems to be acceptably good for both IgG and IgM ($\geq 97\%$ correctly reported negative results), there may be problems with the diagnostic sensitivity. Although sample preparation and shipping was improved (normal serum for diluting instead of PBS, freeze-drying of samples before shipping), the number of correctly IgG positive results increased only from 64% (PEP) to 76%

(EQA). Even worse, only 53% of IgM-positive samples were correctly diagnosed as hantavirus positive. This indicates a risk of overlooking acute infections in patients with early hantavirus infection. However, it should be noted that the majority of the serum samples (17/20) were pre-diluted before the distribution, which resulted in low antibody titre when assayed. This may explain, at least partially, the generally low sensitivities observed. The results regarding the hantavirus strain given by the different laboratories, might be of some importance for the physicians because of the different pathogenic cause for the disease. According to expert opinions a differentiation of the hantavirus strains require the neutralisation test which was not performed. Therefore, we suggest that these kind of information have to be further analysed before given to the physicians and were not considered in this QA.

Overall, in-house and commercial test systems performed almost equally, and also the test protocol (IB, EIA or IFA) seemed to have only little influence on the diagnostic output. However, further EQA runs may help to differentiate between the tests and pinpoint problems with single test systems. There is a clear need to improve most of these assays for a better diagnostic performance in the future.

Summing up, external quality control measures are not only important for 'common' viral pathogens, e.g. HIV, but also for viruses, that are only rarely suspected. To guarantee a continuous quality of these diagnostics, to improve existing test systems, and to ensure the reliability of

diagnostic results, regular EQA runs are an essential tool.

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References

- Brus Sjölander KB, Elgh F, Kallio-Kokko H, Vapalahti O, Hägglund M, Palmcrantz V, Juto P, Vaheri A, Niklasson B, Lundkvist Å. Evaluation of serological methods for diagnosis of Puumala hantavirus infection (nephropathia epidemica). *J Clin Microbiol* 1997;35:3264–8.
- Lewis SM. Quality assurance programmes in the United Kingdom. *Annali Delli Istituto Superiore Di Sanita* 1995;31:53–9.
- Muir P, Ras A, Klapper PE, Cleator GM, Korn K, Aepinus C, Fomsgaard A, Palmer P, Samuelsson A, Tenorio A, Weissbrich B, van Loon AM. Multicenter quality assessment of PCR methods for detection of enteroviruses. *J Clin Microbiol* 1999;37:1409–14.
- Schweiger B, Pauli G, Zeichhardt H, Kuecherer C. A multi-centre quality assessment study to monitor the performance of HIV-1 PCR. *J Virol Meth* 1997;67:45–55.
- Wood WG. Immunoassay external quality assessment in the Federal Republic of Germany. *Annali Delli Istituto Superiore Di Sanita* 1991;27:495–8.