SUPPLEMENT ARTICLE

Development of an Immunofiltration-Based Antigen-Detection Assay for Rapid Diagnosis of Ebola Virus Infection

Andreas Lucht,[1,6] Pierre Formenty,[5] Heinz Feldmann,[6,7] Marion Götz,[1] Eric Leroy,[8] Pierre Bataboukila,[9] Allen Grolla,[6] Friederike Feldmann,[6] Tatiana Wittmann,[8] Patricia Campbell,[12] Catherine Atsangandoko,[10] Paul Boumandoki,[11] Ernst-Jürgen Finke,[1] Peter Miethe,[2] Stephan Becker,[3,4] and Roland Grunow[1,4]

[1]Bundeswehr Institute of Microbiology, Munich, [2]Senova GmbH, Jena, [3]Institute for Virology, Philipps-Universität Marburg, Marburg, and [4]Robert Koch-Institut, Berlin, Germany; [5]Global Outbreak and Alert Response Team, Department of Communicable Diseases Surveillance and Response, World Health Organization, Geneva, Switzerland; [6]Special Pathogens Program, National Microbiology Laboratory, Public Health Agency of Canada, and [7]Department of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada; [8]Service Maladies Emergentes, Centre Internationale Recherches Médicales de Franceville, Franceville, Gabon; [9]Service de Bactériologie, Laboratoire National de Santé Publique, [10]Ecosystèmes Forestiers d'Afrique Centrale (ECOFAC) Congo, and [11]Ministère de la Santé et des la Population, Brazzaville, Republic of the Congo; [12]Médecins Sans Frontières, Amsterdam, The Netherlands

Ebola virus (EBOV) has caused outbreaks of severe viral hemorrhagic fever in regions of Central Africa where medical facilities are ill equipped and diagnostic capabilities are limited. To obtain a reliable test that can be implemented easily under these conditions, monoclonal antibodies to the EBOV matrix protein (VP40), which previously had been found to work in a conventional enzyme-linked immunosorbent assay, were used to develop an immunofiltration assay for the detection of EBOV antigen in chemically inactivated clinical specimens. The assay was evaluated by use of defined virus stocks and specimens from experimentally infected animals. Its field application was tested during an outbreak of Ebola hemorrhagic fever in 2003. Although the original goal was to develop an assay that would detect all EBOV species, only the Zaire and Sudan species were detected in practice. The assay represents a first-generation rapid field test for the detection of EBOV antigen that can be performed in 30 min without electrical power or expensive or sensitive equipment.

Four different species of the genus *Ebolavirus* (EBOV), family *Filoviridae,* have been identified: *Zaire ebolavirus* (ZEBOV), *Sudan ebolavirus* (SEBOV), *Cote d'Ivoire ebolavirus* (CIEBOV), and *Reston ebolavirus* (REBOV) [1]. SEBOV and ZEBOV cause a severe form of viral hemorrhagic fever (VHF), with case-fatality rates ranging from 53% to 90%. In contrast, CIEBOV has been implicated in only a single clinical case of Ebola hemorrhagic fever (EHF), and REBOV seems to have low virulence in humans [2, 3].

At present, diagnostics for acute filoviral infection are based mainly on reverse-transcription polymerase chain reaction (RT-PCR) technology and antigen-capture ELISA, which can be supplemented with antibody-detection assays [3–5]. These assays are established in national and international reference laboratories equipped with the necessary biocontainment. However, rapid and less-sophisticated methods are urgently needed for diagnostics under rural or technically demanding conditions in the field. In addition, cultural beliefs make the collection of blood samples difficult in many communities. Therefore, diagnostic tests that use more–culturally acceptable clinical specimens, such as urine samples, are highly desirable.

Potential conflicts of interest: P.M. is an employee of Senova GmbH; all other authors: none reported.

Presented in part: Filoviruses: Recent Advances and Future Challenges, International Centre for Infectious Diseases Symposium, Winnipeg, Manitoba, Canada, 17–19 September 2006.

Financial support: Bundesministerium der Verteidigung (Sonderforschungsauftrag 23Z1-S-439902), Deutsche Forschungsgemeinschaft (Sonderforschungsbereiche 593 [B3] and 535 [A13]), Canadian Institutes of Health Research (MOP-43921), and Public Health Agency of Canada. Supplement sponsorship is detailed in the Acknowledgments.

Reprints or correspondence: Dr. Andreas Lucht, Pivitsteich 15, D-32791 Lage, Germany (andreaslu@gmx.de).

The Journal of Infectious Diseases 2007;196:S184–92
© 2007 by the Infectious Diseases Society of America. All rights reserved.
0022-1899/2007/19610S2-0011$15.00
DOI: 10.1086/520593

The goal of this study was the development of a rapid, safe, and reliable antigen-detection assay for use with blood and urine samples. The assay was based on the immunofiltration technology that uses previously characterized EBOV-specific monoclonal antibodies (MAbs) that are directed against matrix protein VP40 of all known EBOV species and that previously had been found to work in a conventional ELISA [6]. In this newly developed assay, 1 MAb binds to the column matrix to immobilize EBOV VP40; the second MAb is biotin labeled and used for detection of the bound viral antigen. VP40 is one of the most abundant proteins in viral particles [7] and, therefore, is an appropriate target for antigen-detection assays.

MATERIALS AND METHODS

Viral antigens. Virus strains ZEBOV Mayinga, SEBOV Boniface, CIEBOV Cote d'Ivoire, REBOV Reston, and Marburg virus (MARV) Musoke and Angola were grown in Vero E6 cells at biosafety level (BSL) 4 [8]. The infectious doses for the virus stock were as follows: 4×10^7 $TCID_{50}$/mL for ZEBOV, 1×10^5 $TCID_{50}$/mL for SEBOV, 1×10^6 $TCID_{50}$/mL for REBOV, and 5×10^6 $TCID_{50}$/mL for MARV Musoke and Angola. We were unable to determine a titer for CIEBOV because this virus stock did not produce a visible cytopathogenic effect and because a focus-forming unit assay has not been developed. However, on the basis of an RT-PCR assay using generic EBOV primers, the titer was estimated to be ~1 $\log_{10}$ lower than that for REBOV (i.e., $\sim 1 \times 10^5$ $TCID_{50}$/mL). Virus stocks were inactivated either by boiling for 10 min in 1% SDS or by γ-irradiation (10 Mrad) and were used to spike human serum and urine samples. In addition, antigen was prepared from infected animals. Female BALB/c mice (5 weeks of age) were infected intraperitoneally with 1.5×10^3 pfu of mouse-adapted ZEBOV [9], at BSL4; mock-infected BALB/c mice served as control animals. Before the mice were killed, blood was collected in EDTA anticoagulant tubes, by cardiac puncture, from anesthetized animals on days 2, 4, and 5 after infection, and virus was inactivated by γ-irradiation (10 Mrad). All animal experiments were performed in Winnipeg at the National Microbiology Laboratory, Public Health Agency of Canada, in accordance with institutional guidelines and following the guidelines of the Canadian Council on Animal Care, Ottawa.

MAbs. Murine hybridoma cell lines producing MAbs directed against ZEBOV antigen had been used previously in an ELISA format [6]. On the basis of previous characterization, MAbs 5F6 and 2C4 were selected for large-scale production in the Tecnomouse system (Integra Biosciences) using protein-free medium supplemented with high glucose (4.5 g/L), high glutamine (4 mmol/L), and 150 mg/L gentamycin (all provided by Life Technologies). The purification of MAbs by protein G was done by use of the MAb Trap GII kit (Amersham Pharmacia). Subsequently, MAb 5F6 was labeled with biotin by use of a biotin labeling kit (Boehringer Mannheim).

Clinical specimens. Clinical specimens were collected during an EHF outbreak in Mbomo and Mbanza, Republic of the Congo (RC), in December 2003 [10]. Samples were taken from 2 case patients with EHF (at death at days 7 and 8 after onset of symptoms; cases confirmed by RT-PCR analysis and antigen-capture ELISA), 13 patients with suspected cases of EHF (at days 6–20 after onset of symptoms), and 5 asymptomatic contacts (table 1). In total, the specimens collected included 20 serum, 14 urine, 5 saliva, and 2 sweat samples and 1 tear sample. In addition, 99 serum and 104 urine samples from healthy German donors and 80 serum samples from healthy African donors (provided by Herbert Schmitz, Bernhard-Nocht-Institut, Hamburg) were used as negative control samples. Specimens were inactivated for serological testing by the addition of SDS to a final concentration of 1% and for RT-PCR analysis as described below (see description of RNA isolation in subsection "Confirmatory assays").

Immunofiltration assay. A detailed description of the assay and of test performance is provided in the Appendix, which appears only in the online edition of the *Journal.* In brief, the column matrix was coated with 750 μL of MAb 2C4 (20 μg/mL in a carbonate buffer of 0.1 mol/L Na_2CO_3 and $NaHCO_3$ [pH 9.0]). Serum specimens were incubated for 30 min with 1% SDS and diluted 1:4 (vol/vol) in sample dilution buffer (0.01 mol/L PBS, 5% bovine serum albumin [BSA], and 0.05% Tween 20). Urine specimens were mixed 9:1 (vol/vol) with 10× urine buffer (0.1 mol/L PBS, 50% BSA, and 0.5% Tween 20) and cleared through a 1.2-μm syringe filter (Sartorius). The diluted specimens were applied to the column matrix. The matrix was washed with 750 μL of washing buffer (0.01 mol/L PBS, 0.1% BSA, 0.05% Tween 20, and 0.1% 5-bromo-5-nitro-1,3-dioxane [BND]) and incubated with 500 μL of biotin-labeled MAb 5F6 (10 μg/mL, diluted in 100 mmol/L potassium phosphate buffer, 1.5% casein, 0.05% Tween 20, and 0.1% BND), followed by 500 μL of streptavidin–horseradish peroxidase (HRP) 40 (2 μg/mL [Senova], diluted in 100 mmol/L potassium phosphate buffer, 1.5% casein, and 0.05% Tween 20). The column was washed 3 times (twice with washing buffer and once with a substrate buffer of 100 mmol/L NaCl and 0.03% BND), and HRP detection was initiated by the addition of 500 μL of TMB (3,3′,5,5′-tetramethylbenzidine membrane peroxidase substrate [Kirkegaard and Perry Laboratories]). The entire assay was performed in ~30 min, with all reagents supplied ready to use.

Confirmatory assays: IgG ELISA, antigen-detection ELISA, and RT-PCR. All clinical specimens collected in Mbomo and Mbanza were shipped to Franceville, Gabon, where they were tested for EBOV-specific antigens and antibodies by use of established assays based on an ELISA format [11–13]. For the

Table 1. Laboratory results from the investigation of an outbreak of Ebola hemorrhagic fever in Mbomo, Republic of the Congo, 2003.

Patient	Sex	Age, years	Case classification	Day after onset	Immunofiltration assay		RT-PCR[a]	Antigen-detection ELISA[b]	Antibody-detection ELISA[b]	Result
					OD	Interpretation				
1	F	5	Probable	7	2.158	+	NT	+	–	Confirmed case
2	M	22	Probable	8	1.671	+	+	+	–	Confirmed case
3	M	19	Suspected	6	.223	–	–	–	–	Not a case
4	M	1	Suspected	8	.260	–	–	–	–	Not a case
5	M	6	Suspected	10	.218	–	–	–	+	Convalescent phase
6	M	31	Suspected	10	.150	–	–	–	+	Convalescent phase
7	M	26	Suspected	14	.078	–	–	–	–	Not a case
8	F	23	Suspected	15	.141	–	–	–	–	Not a case
9	F	18	Suspected	15	.259	–	–	–	+	Convalescent phase
10	F	22	Suspected	16	.065	–	–	–	+	Convalescent phase
11	F	20	Suspected	16	.108	–	–	–	–	Not a case
12	F	40	Suspected	18	.190	–	–	–	–	Not a case
13	F	14	Suspected	18	.128	–	–	–	–	Not a case
14	F	22	Suspected	20	.299	–	–	–	+	Convalescent phase
15	F	38	Suspected	20	.084	–	–	–	–	Not a case
16	M	?	Contact	…	.145	–	–	–	–	Not a case
17	F	?	Contact	…	.241	–	–	–	–	Not a case
18	M	43	Contact	…	.127	–	–	–	–	Not a case
19	F	?	Contact	…	.073	–	–	–	–	Not a case
20	M	?	Contact	…	.094	–	–	NT	NT	Not a case

NOTE. NT, not tested; OD, optical density; RT-PCR, reverse-transcription polymerase chain reaction.

[a] Analysis was done in the field by the authors.

[b] Assays were done at the Centre Internationale Recherches Médicales de Franceville in Franceville, Gabon.

RT-PCR analysis, RNA from both cell culture–derived material and clinical specimens (140 μL) was isolated by use of a viral RNA minikit from Qiagen, in accordance with the manufacturer's instructions. Specimens/samples were analyzed by use of generic filovirus RT-PCR targeted to the nucleoprotein and polymerase genes, by use of the SYBR Green I amplification kit (Roche), as described elsewhere [5].

RESULTS

Assay sensitivity. The general assay cutoff was set to an OD_{510} of 0.4, calculated on the basis of the mean for the 99 negative serum samples from healthy German donors (OD, 0.134) plus 3 SD (OD, 0.094). The sensitivity of the immunofiltration assay was determined by use of ZEBOV Mayinga antigen serially diluted in human serum or urine samples in the absence or presence of 1% SDS. The detection limit of the assay in the absence of SDS was 1×10^5 and 2×10^5 $TCID_{50}$/mL for urine and serum, respectively. In the presence of 1% SDS and 5% BSA (sample dilution buffer), the assay showed greater sensitivity, with a detection limit of 1.25×10^4 $TCID_{50}$/mL for both specimen sources (figure 1*A*). The sensitivity of the assay was further evaluated by comparison of our ZEBOV Mayinga antigen (virus stock, 4×10^7 $TCID_{50}$/mL) with a positive control antigen from an antigen-detection ELISA (provided by T. G. Ksiazek and P. E. Rollin, Centers for Disease Control and Prevention, Atlanta). Both specimens were run in parallel in the presence of 1% SDS and 5% BSA, and the serial dilution was 1:2 in the immunofiltration assay (figure 1*B*). Both antigens were equally well detected in this assay, and the detection limit was $\sim 1.5 \times 10^4$ $TCID_{50}$/mL.

The sensitivity of an assay could become more important for clinical specimens in which antigen might be diluted because of a larger volume. Accordingly, the immunofiltration system was evaluated for the detection of ZEBOV and MARV antigen in 10-mL urine samples. Prior to use, urine samples were filtered, to avoid clogging the column with urine sediment. Only ZEBOV was detected in the urine samples, and no signal was detected from samples containing MARV. The use of larger sample volumes significantly increased the optical density values (ZEBOV antigen) without leading to a higher background (MARV antigen; figure 2). Thus, the use of larger volumes of urine sample can maximize the sensitivity of the assay.

Assay specificity. To determine the specificity of the assay, negative control serum samples (99 from German and 80 from African donors) and urine samples (104 from German donors) were spiked with ZEBOV (1×10^5 $TCID_{50}$/mL) and were com-

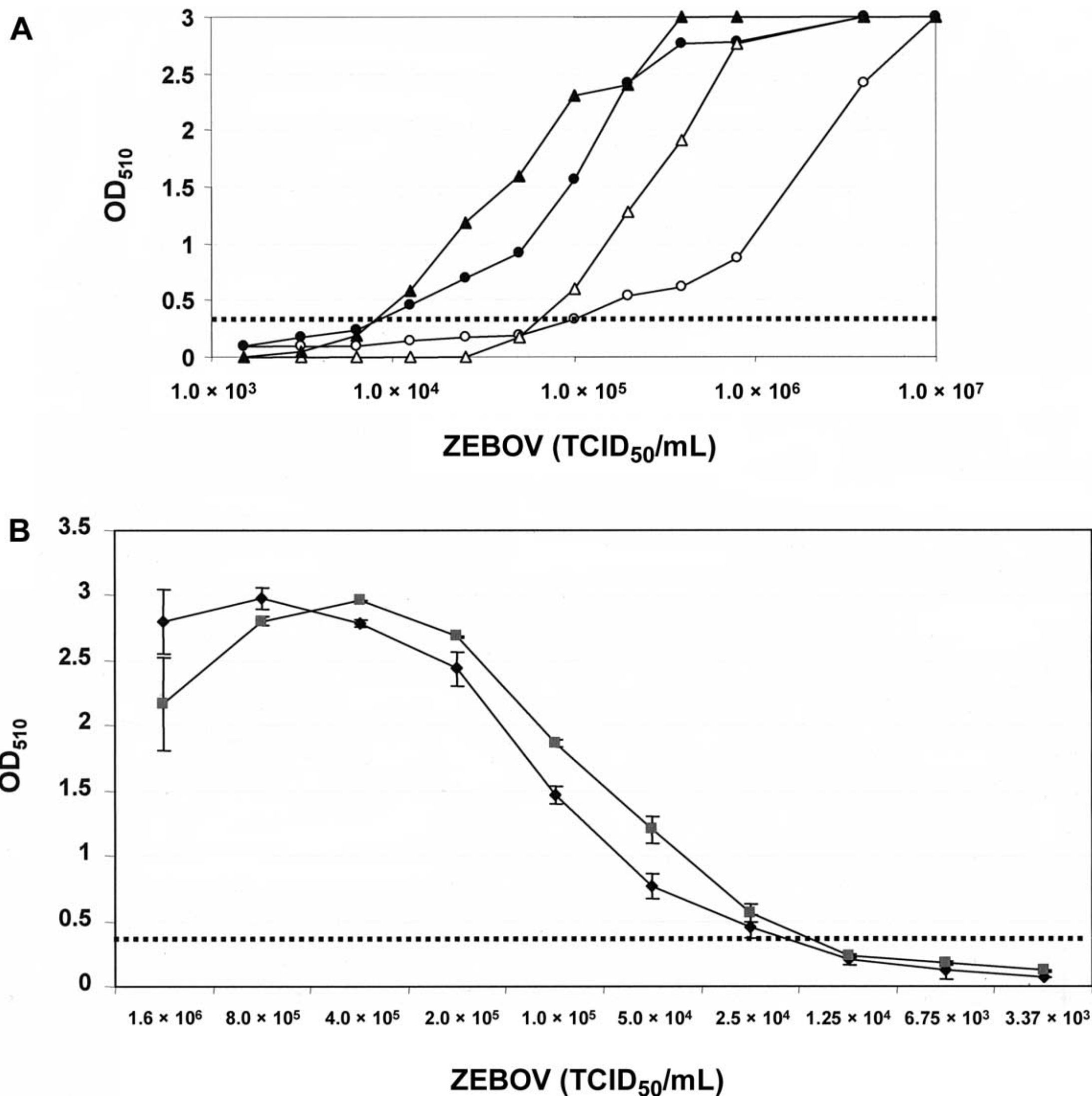

Figure 1. Results of sensitivity testing. *A,* Influence of inactivation, as shown by line plot of titration of γ-inactivated *Zaire ebolavirus* (ZEBOV) Mayinga antigen diluted in serum or urine samples with and without the addition of 1% SDS. *Open circles,* ZEBOV in serum; *filled circles,* ZEBOV in serum with 1% SDS; *open triangles,* ZEBOV in urine; *filled triangles,* ZEBOV in urine with 1% SDS and 5% bovine serum albumin (BSA). *B,* Line plot of a comparative titration in the immunofiltration assay. Comparison was between γ-inactivated ZEBOV Mayinga antigen *(diamonds)* and a defined positive control antigen from a widely used antigen-detection ELISA (*squares;* provided by T. G. Ksiazek and P. E. Rollin, Centers for Disease Control and Prevention, Atlanta). Both antigens were treated with 1% SDS and 5% BSA prior to dilution. The dotted lines indicate the cutoff of the assays, as determined by the mean optical density of 99 negative control serum samples plus 3 SD.

pared with identical, unspiked samples. The mean value and SD were calculated for each group. The viral antigen was well detected in the spiked serum and urine samples. Statistical analysis was performed by use of Student's *t* test. Significantly higher optical density values were found for the spiked samples, compared with those for the corresponding unspiked samples ($P < .01$). No significant differences were detected between the unspiked serum samples from the German and African donors, whereas the slightly higher optical density values for the spiked serum samples from African donors might be explained by hemolysis after the repeated freezing and thawing of these samples, which could lead to reduced flow velocity and therefore a longer incubation time on the column (figure 3*A*).

Subsequently, the immunofiltration assay was tested for its cross-reactivity to antigen derived from different EBOV species, as well as from MARV. In immunoblot assays, MAbs 5F6 and 2C4 had previously been shown to be cross-reactive with strains of all 4 known EBOV species but not with MARV [6]. Thus, different levels of cross-reactivity were expected with the different EBOV species but not with the MARV antigens. Preparations of viral antigen were treated with 1% SDS and 5% BSA and were run either undiluted or diluted in sample buffer.

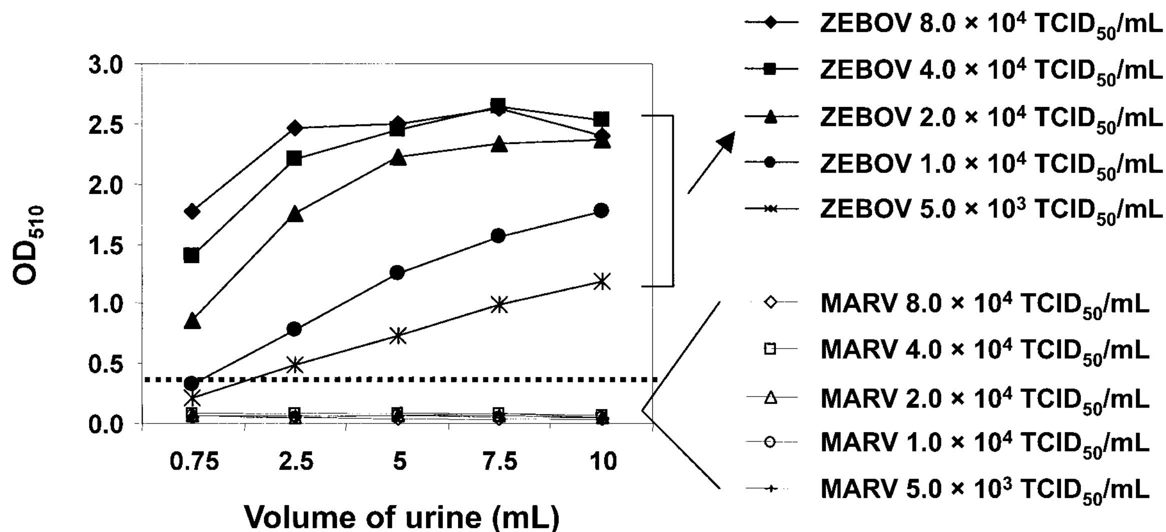

Figure 2. Sensitivity testing using larger volumes of urine samples. Dilutions of *Zaire ebolavirus* (ZEBOV) Mayinga and *Lake Victoria marburgvirus* (MARV) Musoke antigens (previously treated with 1% SDS and 5% bovine serum albumin) in different volumes of urine samples were loaded on the column for antigen detection. An increase in the volume of urine spiked with MARV antigen did not cause false-positive results. The dotted line indicates the cutoff of the assays, as determined by the mean optical density of 99 negative control serum samples plus 3 SD.

The detection limits for SEBOV, REBOV, and CIEBOV antigens were $<1 \times 10^5$ $TCID_{50}$/mL, 1×10^6 $TCID_{50}$/mL, and $>1 \times 10^5$ $TCID_{50}$/mL, respectively (figure 3*B*) and, thus, were 0.5–2 $\log_{10}$ higher than those for ZEBOV antigen (figures 1 and 3*B*). Although the MAbs were able to recognize VP40 of REBOV and CIEBOV, the assay effectively failed to detect these viruses. Results remained negative for all MARV antigens (for strain Musoke, see figure 3*B;* for strain Angola, data not shown) even when the antigens were applied undiluted, indicating no cross-reactivity with antigens from a different genus of the family *Filoviridae.*

Assay application. To evaluate the usefulness of the immunofiltration assay on clinical specimens, we first tested serum samples from experimentally infected mice. The blood specimens were inactivated by γ-irradiation and run in the immunofiltration assay at starting dilutions of 1:100. ZEBOV antigen was barely detectable on day 2 after infection, with increasing levels of viremia on the following days. The day 4 specimen remained antigen positive to a dilution of 10^{-5}, indicating viremia levels of $\sim 10^9$ $TCID_{50}$/mL (data not shown).

The assay was first tested on human specimens during an outbreak of EHF in Central Africa in 2003 [10]. A limited number of human specimens (serum, urine, saliva, sweat, and tears) were collected from case patients with probable EHF (subsequently confirmed by RT-PCR and antigen-detection ELISA), case patients with suspected EHF, and contacts in the families and community. The immunofiltration assay detected EBOV antigen in blood samples, obtained by cardiac puncture, from 2 deceased case patients with probable EHF (table 1 and figure 4). All clinical samples, including serum, urine, saliva, sweat, and tear specimens, obtained from case patients with suspected EHF and from their contacts were negative for EBOV antigen even at a dilution as low as 1:4. To further confirm the results of the immunofiltration assay, all clinical specimens were tested by RT-PCR analysis in the field or by antigen- and antibody-detection ELISAs performed at the Centre Internationale Recherches Médicales de Franceville in Franceville (table 1). Confirmatory testing fully supported the laboratory results obtained by the immunofiltration assay (table 1). Subsequent sequence determination of the amplicon of the only PCR-positive sample (table 1, patient 2; figure 4) identified the causative agent as a strain of ZEBOV. Epidemiological follow-up investigations of the laboratory-tested case patients with suspected EHF or of the contacts did not reveal any EHF symptoms or unexplained deaths, further supporting the laboratory test results (table 1).

DISCUSSION

The development of this new immunofiltration assay for the detection of EBOV antigen will provide a novel and long-awaited tool for future field response to outbreaks of EHF. Despite being less sensitive than RT-PCR analysis, the immunofiltration assay demonstrated a sensitivity similar to that of the widely used antigen-detection ELISA [11, 12], but it is rapid and far less prone to technical complications. The detection of ZEBOV antigen in specimens obtained from animals and humans demonstrates its usefulness in field diagnosis of EHF in humans, as well as the detection of EBOV in such animal species as the great apes.

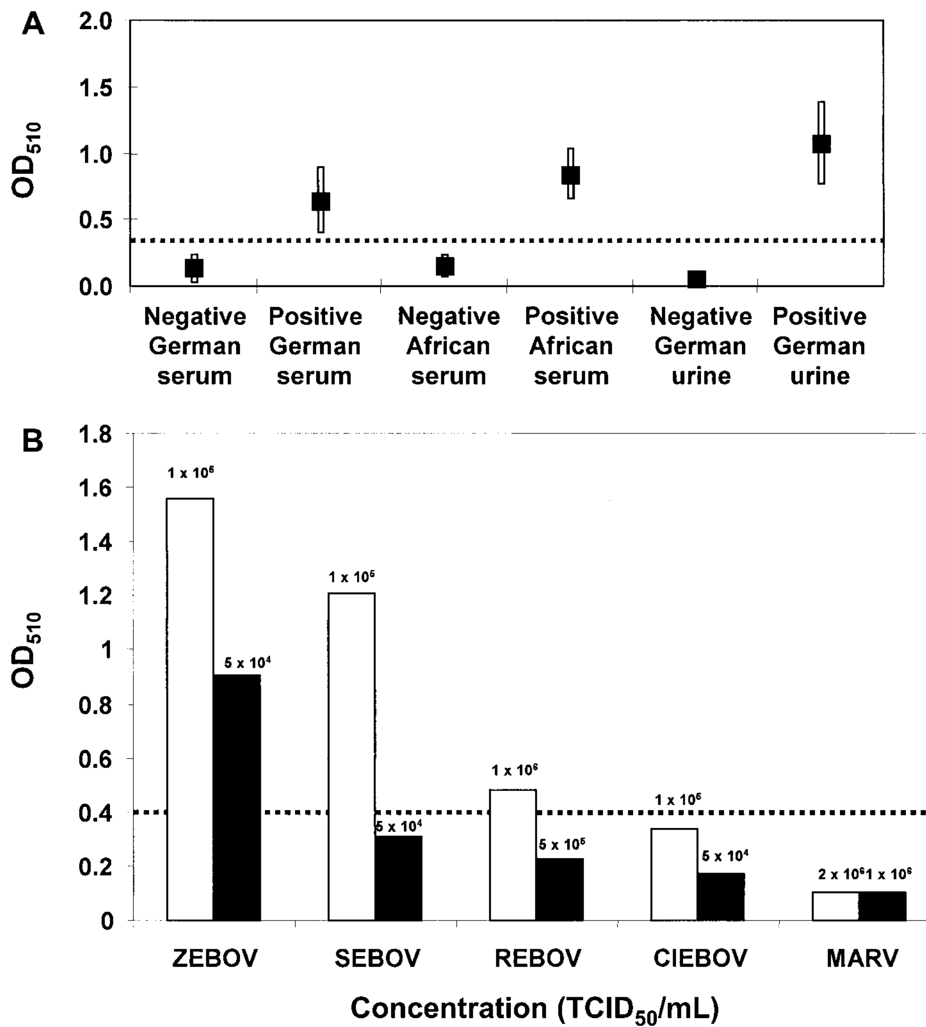

Figure 3. Results of specificity testing. *A,* Comparison of spiked and unspiked serum and urine samples. To test the specificity of the immunofiltration assay, negative control serum samples from 99 German and 80 African donors, as well as urine samples from 104 German donors, were spiked with *Zaire ebolavirus* (ZEBOV) Mayinga (~1×10^5 $TCID_{50}$/mL), and results were compared with those for corresponding unspiked samples. The mean values (± 1 SD) are shown for each group of spiked and unspiked samples. Statistical analysis revealed significantly higher values for the spiked serum and urine samples, compared with the unspiked samples. *B,* Cross-reactivity with antigens for other *Ebolavirus* (EBOV) species and *Lake Victoria marburgvirus* (MARV) Musoke. Different preparations of EBOV and MARV antigens (5×10^4 to 2×10^6 $TCID_{50}$/mL) were applied to the column in the presence of 1% SDS. Open bars indicate high antigen concentrations, and filled bars indicate diluted antigen concentrations (1:2 in sample buffer); numbers above the bars indicate $TCID_{50}$/mL. The dotted lines indicate the cutoff of the assays, as determined by the mean optical density of 99 negative control serum samples plus 3 SD. CIEBOV, *Cote d'Ivoire ebolavirus* Cote d'Ivoire; REBOV, *Reston ebolavirus* Reston; SEBOV, *Sudan ebolavirus* Boniface.

The assay was based on a technique that had been evaluated previously for the detection of antibodies [14] and serum proteins [15]. In this study, we demonstrated for the first time the applicability of the immunofiltration system to the detection of viral antigen. The assay is specific for ZEBOV antigen but also detected SEBOV antigen at a slightly lower sensitivity. Only weak or no cross-reactivity was observed with REBOV and CIEBOV antigens (figure 3*B*). With the exception of a single case of CIEBOV infection [16], all cases of EHF in humans have been caused by ZEBOV or SEBOV, making these 2 EBOV species the most important in terms of public health response.

During the symptomatic stage of EHF, viremia levels can exceed 10^6 pfu/mL, with peak levels up to 10^8 or 10^9 pfu/mL [17]. Thus, it seems reasonable to conclude that the immunofiltration assay developed here, with detection limits of 1.25×10^4 $TCID_{50}$/mL for ZEBOV and $<1 \times 10^5$ $TCID_{50}$/mL for SEBOV ($TCID_{50}$ and plaque-forming units may differ by ~2–10-fold [authors' unpublished data]), will be sufficiently sensitive for diagnosis of a case of ZEBOV or SEBOV infection in a symptomatic patient. This conclusion is supported by the successful use of the assay to diagnose EHF in patients during the outbreak in the RC in 2003 [18], as well as the detection

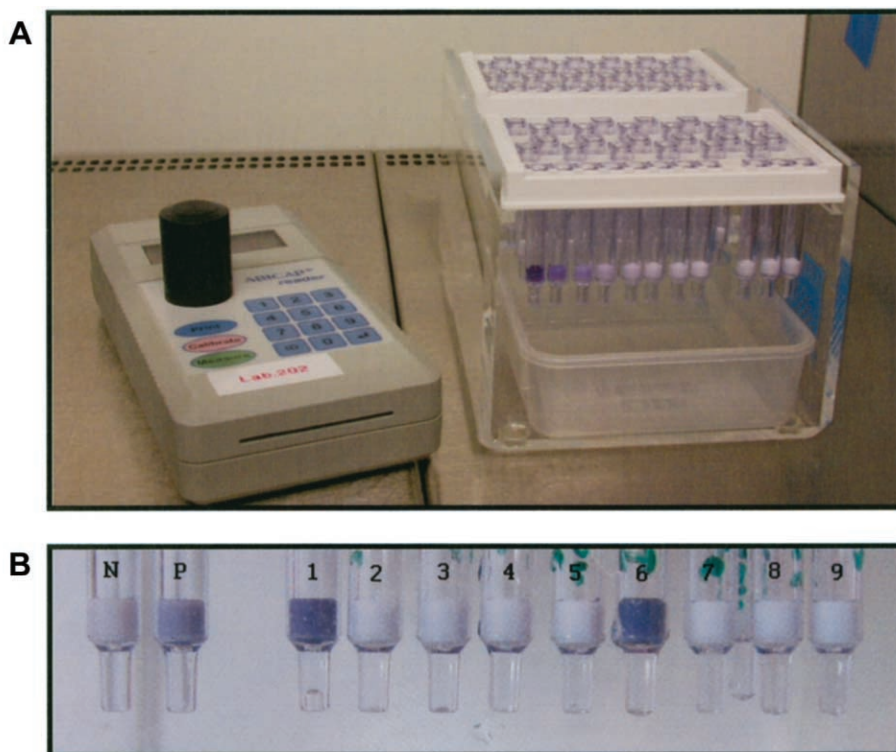

Figure 4. Immunofiltration assay. *A,* Photometer and rack with immunofiltration columns as used in the field. *B,* Results of a representative field test from Mbomo, Republic of the Congo. Patient serum samples are in columns 1–9. Samples 1 and 6 were positive for *Zaire ebolavirus* (ZEBOV) antigen (see also table 1, patients 1 and 2); all other serum samples were negative for ZEBOV antigen. The visual results were confirmed by photometric analysis. N, negative control serum sample; P, ZEBOV Mayinga as a positive control.

of ZEBOV antigen in experimentally infected animals starting on day 2 after infection (this study). The usefulness of this assay in the detection of REBOV and CIEBOV in clinical specimens is questionable, but high viremia levels, which could be expected during infection, may overcome the lower sensitivity for REBOV and CIEBOV and result in a detectable reaction.

Biosafety is an important consideration in the diagnosis of VHFs such as EHF, particularly in the field. Therefore, diagnostics should be performed on inactivated specimens. Not only did the immunofiltration assay described here perform well with SDS-treated and thus inactivated serum and urine samples, but its performance was superior when SDS-treated samples were used, compared with when native (untreated) clinical samples were used. This is clearly an advantage over currently used antigen-detection assays based on an ELISA format. However, it should be remembered that BSA needs to be added to SDS-treated clinical specimens with low endogenous protein concentrations, such as urine and saliva samples. With such low protein concentrations, SDS might denature the MAbs.

During past EHF outbreaks in Gabon and the RC [19, 20], the population has become increasingly reluctant about blood sampling, because of cultural beliefs, fear of witchcraft, and fear of infection caused by invasive manipulations such as bleeding. For example, cooperation with international teams ceased completely when the teams were accused of being the source of the infection. In 2002, a similar belief in the population forced a World Health Organization team to leave the region of an outbreak investigation [21]. In addition, nosocomial infections mainly caused by the reuse of contaminated needles and syringes are a well-documented and well-known infection route during EHF outbreaks, leading to objections in the population to invasive procedures such as venipuncture and vaccination [22]. Currently, aid agencies and response personnel are looking into alternative clinical specimens for laboratory diagnosis that are obtained by noninvasive procedures and, thus, are safer [23]. In the past, EBOV had been detected in vaginal, rectal, and conjunctival swabs, as well as in seminal fluid [24]. Urine samples are likely to be more acceptable to these populations; however, the use of urine samples to diagnose EBOV infection in humans is not established. Urine samples from primates experimentally infected with EBOV contained virus concentrations up to 10^3–10^5 pfu/mL [25]. In addition, epidemiological studies suggest that contact with urine might play a role

in human-to-human transmission [26]. Using the immunofiltration assay, we were able to detect ZEBOV antigen in spiked human urine samples, with a sensitivity of $\sim 1.25 \times 10^4$ pfu/mL. Since the column format allows for the application of larger volumes (up to 10 mL), the sensitivity of the assay may be sufficiently high, even though virus concentrations in urine are, in general, lower than those in serum.

Finally, the experience with the immunofiltration assay during the EHF outbreak in the RC in 2003 demonstrated its field applicability in an extremely remote, infrastructure-poor geographic region. Using this assay, we were able to correctly diagnose 2 EHF cases and to exclude suspected case patients and their contacts, confirmed by the current reference-standard assays for diagnosis of acute EBOV infection, namely, RT-PCR analysis and antigen-detection ELISA. Thus, during this outbreak, field diagnostics played an important role in case patient management and contact tracing. The rapid provision of results to the community may actually lessen cultural fears of invasive procedures such as venipuncture, as may have been the case during the EHF outbreak in Mbomo, where venipuncture was much more accepted [27]. A more extensive evaluation of this assay under field conditions that include a larger number of specimens is still needed, but the current results are quite promising.

In conclusion, a rapid immunofiltration assay was developed for the detection of EBOV antigen in the field. The assay allowed for simple inactivation of specimens by SDS, and accurate results were provided in 30 min without the need for electrical power or technically demanding equipment. Owing to its simplicity, the local staff was easily trained to perform this assay. With the possibility of future treatment options for patients with EHF [28–33], early on-site diagnosis and follow-up becomes even more important. This test format may fill a gap in the management of future EHF outbreaks and may be adaptable for the detection of MARV infection, as well as infections caused by other related pathogens. In addition, the assay has potential for application with wildlife species, particularly the great apes, that are affected by outbreaks of EBOV infection in Central Africa [16, 34].

Acknowledgments

We thank Ella Zeman (Bundeswehr Institute of Microbiology, Munich) and Daryl Dick and Michael Garbutt (National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg) for their excellent technical assistance. We are very thankful to all those colleagues who supported our work in Mbomo, especially Phillippe Yaba, Sebastiao Nkunku, André Salemo, Alain Epelboin, Derek Hardy, Kamel Ait-Ikhlef, and Gerard Eon.

Supplement sponsorship. This article was published as part of a supplement entitled "Filoviruses: Recent Advances and Future Challenges," sponsored by the Public Health Agency of Canada, the National Institutes of Health, the Canadian Institutes of Health Research, Cangene, CUH2A, Smith Carter, Hemisphere Engineering, Crucell, and the International Centre for Infectious Diseases.

References

1. Feldmann H, Geisbert TW, Jahrling PB, et al. Filoviridae. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA, eds. Virus taxonomy. Eighth report of the International Committee on Taxonomy of Viruses. London: Elsevier/Academic Press, **2004**:645–53.
2. Feldmann H, Jones S, Klenk HD, Schnittler HJ. Ebola virus: from discovery to vaccine. Nat Rev Immunol **2003**; 3:677–85.
3. Sanchez A, Feldmann H. Filoviridae: Marburg and Ebola viruses. In: Knipe DM, Howley PM, eds. Fields virology. Vol 2, 5th ed. Philadelphia: Lippincott Williams & Wilkins, **2006**:1409–48.
4. Grolla A, Lucht A, Dick D, Strong JE, Feldmann H. Laboratory diagnosis of Ebola and Marburg hemorrhagic fever. Bull Soc Pathol Exot **2005**; 98:205–9.
5. Strong JE, Grolla A, Jahrling PB, Feldmann H. Filoviruses and arenaviruses. In: Detrick B, Hamilton RG, Folds JD, eds. Manual of molecular and clinical laboratory immunology. Washington, DC: ASM Press, **2006**:774–90.
6. Lucht A, Grunow R, Möller P, Feldmann H, Becker S. Development, characterization and use of monoclonal antibodies for the detection of Ebola virus. J Virol Methods **2003**; 111:21–8.
7. Elliott LH, Kiley MP, McCormick JB. Descriptive analysis of Ebola virus proteins. Virology **1985**; 147:169–76.
8. Becker S, Feldmann H, Will C, Slenczka W. Evidence for occurrence of filovirus antibodies in humans and imported monkeys: do subclinical filovirus infections occur worldwide? Med Microbiol Immunol **1992**; 181:43–55.
9. Bray M, Davis K, Geisbert T, Schmaljohn C, Huggins J. A mouse model for evaluation of prophylaxis and therapy of Ebola hemorrhagic fever. J Infect Dis **1998**; 178:651–61.
10. World Health Organization. Outbreak news: Ebola haemorrhagic fever, Republic of the Congo. Wkly Epidemiol Rec **2003**; 78:405.
11. Ksiazek TG, Rollin PE, Jahrling PB, Johnson E, Dalgard DW, Peters CJ. Enzyme immunosorbent assay for Ebola virus antigens in tissues of infected primates. J Clin Microbiol **1992**; 30:947–50.
12. Ksiazek TG, Rollin PE, Williams AJ, et al. Clinical virology of Ebola hemorrhagic fever (EHF): virus, virus antigen, and IgG and IgM antibody findings among EHF patients in Kikwit, Democratic Republic of the Congo, 1995. J Infect Dis **1999**; 179(Suppl 1):S177–87.
13. Ksiazek TG, West CP, Rollin PE, Jahrling JB, Peters CJ. ELISA for the detection of antibodies to Ebola viruses. J Infect Dis **1999**; 179(Suppl 1):S192–8.
14. Schell D. Fast quantification of mouse IgG from cell culture supernatants. In: Spier RE, Griffiths JB, Berthold W, eds. Animal cell technology. Oxford, UK: Butterworth-Heinemann, **1994**:366–8.
15. Hartmann H, Lübbers B, Cararetto M, Bautsch W, Klos A, Köhl J. Rapid quantification of C3a and C5a using a combination of chromatographic and immunoassay procedures. J Immunol Methods **1993**; 166:35–44.
16. LeGuenno B, Formenty P, Wyers M, Gounon P, Walker F, Boesch C. Isolation and partial characterization of a new strain of Ebola virus. Lancet **1995**; 345:1271–4.
17. Towner JS, Rollin PE, Bausch DG, et al. Rapid diagnosis of Ebola haemorrhagic fever by reverse transcription–PCR in an outbreak setting and assessment of patient viral load as a predictor of outcome. J Virol **2004**; 78: 4330–41.
18. Boumandouki P, Formenty P, Epelboin A, et al. Clinical management of patients and deceased during the Ebola outbreak from October to December 2003 in Republic of Congo. Bull Soc Pathol Exot **2005**; 98: 218–23.
19. World Health Organization. Outbreak(s) of Ebola haemorrhagic fever,

Congo and Gabon, October 2001–July 2002. Wkly Epidemiol Rec **2003**; 78:223–8.
20. World Health Organization. Outbreak(s) of Ebola haemorrhagic fever in the Republic of Congo, January–April 2003. Wkly Epidemiol Rec **2003**; 78:285–9.
21. World Health Organization. Outbreak news: Ebola, Gabon and Congo (update). Wkly Epidemiol Rec **2002**; 77:9.
22. Peters CJ, LeDuc JW. An introduction to Ebola: the virus and the disease. J Infect Dis **1999**; 179(Suppl 1):ix–xvi.
23. Formenty P, Leroy EM, Epelboin A, et al. Detection of Ebola virus in oral fluid specimens during outbreaks of Ebola virus hemorrhagic fever in the Republic of Congo. Clin Infect Dis **2006**; 42:1521–6.
24. Rodriguez LL, De Roo A, Guimard Y, et al. Persistence and genetic stability of Ebola virus during the outbreak in Kikwit, Democratic Republic of the Congo, 1995. J Infect Dis **1999**; 179(Suppl 1):S170–6.
25. Fisher-Hoch SP, Platt GS, Neild GH, et al. Pathophysiology of shock and hemorrhage in a fulminating viral infection (Ebola). J Infect Dis **1985**; 152:887–94.
26. Dowell S, Mukunu FR, Ksiazek TG, Khan AS, Rollin PE, Peters CJ. Transmission of Ebola hemorrhagic fever: a study of risk factors in family members, Kikwit, Democratic Republic of the Congo, 1995. J Infect Dis **1999**; 179(Suppl 1):S87–91.
27. Hewlett BS, Epelboin A, Hewlett BL, Formenty P. Medical anthropology and Ebola in Congo: cultural models and humanistic care. Bull Soc Pathol Exot **2005**; 98:230–6.
28. Stroeher U, Feldmann H. Progress towards the treatment of Ebola haemorrhagic fever. Expert Opin Investig Drugs **2006**; 15:1523–35.
29. Feldmann H, Jones SM, Schnittler HJ, Geisbert T. Therapy and prophylaxis of Ebola virus infections. Curr Opin Investig Drugs **2005**; 6: 823–30.
30. Enterlein S, Warfield KL, Swenson DL, et al. VP35 knockdown inhibits Ebola virus amplification and protects against lethal infection in mice. Antimicrob Agents Chemother **2006**; 50:984–93.
31. Geisbert TW, Hensley LE, Jahrling PB, et al. Treatment of Ebola virus infection with a recombinant inhibitor of factor VIIa/tissue factor: a study in rhesus monkeys. Lancet **2003**; 362:1953–8.
32. Paragas J, Geisbert TW. Development of treatment strategies to combat Ebola and Marburg viruses. Expert Rev Anti Infect Ther **2006**; 4:67–76.
33. Bray M, Paragas J. Experimental therapy of filovirus infections. Antiviral Res **2002**; 54:1–17.
34. Leroy EM, Rouquet P, Formenty P, et al. Multiple Ebola virus transmission events and rapid decline of central African wildlife. Science **2004**; 303:387–90.