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Development and Evaluation of a Real-Time Reverse Transcription-Loop-Mediated Isothermal Amplification Assay for Rapid Detection of Rift Valley Fever Virus in Clinical Specimens▽

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This paper reports on the development and validation of a real-time reverse transcription-loop-mediated isothermal amplification assay (RT-LAMP) targeting the genomic large RNA segment of Rift Valley fever virus (RVFV). The set of six designed RT-LAMP primers identified strains of RVFV isolated in geographically distinct areas over a period of 50 years; there was no cross-reactivity with other genetically related and unrelated arboviruses. When testing serial sera and plasma from sheep experimentally infected with wild-type RVFV, there was 100% agreement between results of the RT-LAMP, a TaqMan-based real-time PCR, and virus isolation. Similarly, the assay had very high levels of diagnostic sensitivity and specificity when testing various clinical specimens from humans and animals naturally infected with the virus during recent outbreaks of the disease in Africa. The detection of specific viral genome targets in positive clinical specimens was achieved in less than 30 min. As a highly accurate, rapid, and very simple nucleic acid detection format, the RT-LAMP has the potential to be used in less-well-equipped laboratories in Africa and as a portable device during RVF outbreaks in remote areas, and it can be a valuable tool for the differential diagnosis of viral hemorrhagic fevers.

Rift Valley fever (RVF) is an acute disease of domestic ruminants in Africa and Madagascar, caused by a mosquito-borne virus of the genus *Phlebovirus* of the family *Bunyaviridae* (18). Since RVF virus (RVFV) was first isolated in 1930 in the Rift Valley in Kenya, it periodically causes large epidemics in domestic ruminants, characterized by high levels of mortality among newborns and abortions in pregnant animals (30). Humans become infected through a mosquito bite, by the handling of aborted fetal material, or during the slaughter of infected animals and typically develop a mild, self-limiting febrile disease (30). However, severe illness, including retinitis, hepatocellular failure, acute renal failure, meningoencephalitis, and hemorrhagic manifestations, occurs in a small proportion of patients (10, 12, 13).

Techniques for the diagnosis of RVF include virus isolation, detection of specific antibody responses, and molecular assays. RVFV can be isolated from serum or whole blood during the febrile stage of the disease as well as from the livers, spleens, and brains of animals who have succumbed to the disease or aborted fetuses. Isolation of the virus is achieved in hamsters,

infant or adult mice, and various cell cultures (35). Various serological assays are used to detect antibodies against RVFV, but the virus neutralization test is regarded as the gold standard (23). It is highly accurate, with little or no cross-neutralization with other phleboviruses (33), but since it requires live virus, it can be done only in biocontainment facilities. Enzyme-linked immunosorbent assays, based on inactivated sucrose-acetone-extracted antigens derived from tissue culture or mouse brain, have been extensively validated for the serodiagnosis of RVF (22, 23). However, their production requires biocontainment facilities to limit the risk of exposure of laboratory personnel to infection (9, 29). An indirect enzyme-linked immunosorbent assay based on the recombinant nucleocapsid protein of RVFV has been recently developed for the detection of specific antibodies in human and animal sera (6, 24, 25). Highly sensitive PCR assays for the detection and quantification of RVFV have been reported, including reverse transcriptase PCR (3, 28) and real-time detection PCR (RTD-PCR) based on TaqMan probe technology (2), but they have not been validated for routine diagnostic use.

The loop-mediated isothermal amplification (LAMP) method has been shown to be highly accurate for the detection of DNA (4, 20) and RNA (14, 32, 36) viruses, differentiation of viral serotypes and subtypes (17, 21), and rapid diagnosis of bacterial infections (5). LAMP amplifies target nucleic acid under isother-

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TABLE 1. Primers used for RT-LAMP of the polymerase gene (L segment) of RVFV

Primer description	Primer name	Genome position	Length (bp)	Sequence
Forward outer	F3	4081–4100	20	TGGGGATCTAGG AAGAAGTT
Backward outer	B3	4269–4289	21	GAGGCCATGACTT TACAAACT
Forward inner	F1c	4149–4172	44	AGCACCTCTGGAT TCTCATTTATT
	F2	4102–4121		CAGAAATTGAGA GACCGTTT
Backward inner	B1c	4186–4207	41	AGAACAGGCCCA GAAATATTGT
	B2	4238–4256		GACAATGATGAC ACAACAC
Loop forward	LF	4123–4147	25	GTTCAATCCAGTT CTCTGGTATGTT
Loop backward	LB	4212–4234	23	CATTGCAGAGAA AGTCCATAGCC

mal conditions, usually between 60°C and 65°C (19). Hence, only simple equipment, such as a heating block or water bath, is required, obviating the need for expensive automated thermal cyclers. It relies on autocycling strand displacement DNA synthesis by *Bst* DNA polymerase and a set of four or six primers. Two inner primers and two outer primers define the target region, and an additional set of primers, termed loop primers, can be added to increase the sensitivity of the assay. The final products of the LAMP reaction are DNA molecules with a cauliflower-like structure of multiple loops consisting of repeats of the target sequence. The products can be analyzed by real-time monitoring of the turbidity resulting from the production of magnesium pyrophosphate precipitate during the DNA amplification reaction, by using an intercalating fluorescent dye and visualization by natural light or with the aid of UV irradiation, or by agarose gel electrophoresis (15, 16).

This paper reports the development and diagnostic evaluation of a real-time reverse transcription-LAMP assay (RT-LAMP) targeting the large RNA segment in a wide spectrum of RVFV isolates recovered in the field over a period of 50 years (1944 to 2007) and various types of clinical specimens from sheep experimentally infected with wild-type virus and from humans and animals naturally infected during recent outbreaks of the disease in Africa.

MATERIALS AND METHODS

Design of RVFV-specific RT-LAMP primers and optimization of RT-LAMP. The oligonucleotide primers for RT-LAMP amplification of RVFV were designed from the putative RNA-dependent RNA polymerase gene (L segment). The nucleotide sequence of the prototype strain of RVFV was retrieved from GenBank (accession number X56464) and aligned with the available sequences of other isolates to identify the conserved regions by using CLUSTALW software version 1.83 (DNA Data Bank of Japan; http://clustalw.ddbj.nig.ac.jp/top-e .html). A potential target region was selected from the aligned sequences, and six primers, comprising two outer (F3 and B3), two inner (FIP and BIP), and two loop (LF and LB) primers (where F indicates forward and B indicates backward) that recognize eight distinct regions on the target sequence (Table 1), were designed using LAMP primer software PrimerExplorer V4 (http://primerexplorer .jp/elamp4.0.0/index.html; Eiken Chemical Co., Japan), as described previously by Notomi et al. (19).

To determine the optimal incubation temperature for the RVF RT-LAMP, RNA was extracted from the SPU 22/07 0121 Kenya strain of RVFV (100 pg per reaction and 10 pg per reaction), negative serum, and tissue culture supernatant, and the assay was run using a Loopamp RNA amplification kit (Eiken Chemical Co., Ltd. Tokyo, Japan) as described by the manufacturer, at temperatures ranging from 61°C to 65°C.

Cell culture and viruses. The analytical sensitivity of RT-LAMP was evaluated by testing 17 RVFV strains representing a wide geographic and historic spectrum of isolates recovered in the field over the last 50 years (1944 to 2007) (Table 2), with titers ranging from $10^{5.2}$ 50% tissue culture infective doses ($TCID_{50}$)/ml to $10^{7.3}$ $TCID_{50}$/ml. Five selected isolates representing the phylogenetic spectrum of RVFV, namely, those from West Africa, Egypt, and central, eastern, and southern Africa (Table 2), were tested in the range of 0.0065 to 65,000 $TCID_{50}$ per reaction volume. The analytical specificity of RT-LAMP was evaluated by testing six African phleboviruses related to RVFV, Akabane ($10^{6.8}$ $TCID_{50}$/ml), Bunyamwera ($10^{7.8}$ $TCID_{50}$/ml), Gabek Forest ($10^{7.0}$ $TCID_{50}$/ml), Saint Floris ($10^{5.8}$ $TCID_{50}$/ml), Arumowot ($10^{4.8}$ $TCID_{50}$/ml), and Gordil ($10^{5.8}$ $TCID_{50}$/ml) viruses and six other unrelated arboviruses, yellow fever ($10^{6.0}$ $TCID_{50}$/ml), dengue I ($10^{5.5}$ $TCID_{50}$/ml), West Nile lineage 1 ($10^{7.8}$ $TCID_{50}$/ml), West Nile lineage II ($10^{6.0}$ $TCID_{50}$/ml), chikungunya ($10^{7.5}$ $TCID_{50}$/ml), and Crimean-Congo hemorrhagic fever ($10^{4.3}$ $TCID_{50}$/ml) viruses. All of these virus isolates were obtained from the Special Pathogens Unit of the National Institute for

TABLE 2. Identification, year of isolation, source, and origin of RVFV isolates

Identification	Yr of isolation	Source	Origin	Genetic lineage[a]	Log₁₀ TCID₅₀ titer/ml
SNS UGA44	1944	Smithburn strain	Uganda	C	$10^{5.2}$
Lunyo UGA55	1955	Mosquito	Uganda	C	$10^{6.3}$
ArB1976CAR69	1969	Mosquito	Central African Republic	C	$10^{6.8}$
VRL2373ZI	1974	Bovine	Zimbabwe	C	$10^{6.3}$
H1825RSA75	1975	Human	South Africa	C	$10^{7.0}$
B1143KEN77	1977	Human	Kenya	C	$10^{6.8}$
ZH501EGY77[b]	1977	Human	Egypt	E	$10^{5.8}$
ZH548EGY77	1977	Human	Egypt	E	$10^{6.3}$
VRL1187ZIM78	1978	Bovine	Zimbabwe	C	$10^{7.0}$
Ar811MAD79	1979	Mosquito	Madagascar	E	$10^{6.3}$
Ar20368RSA81[b]	1981	Mosquito	South Africa	C	$10^{6.8}$
ArD38388BF83	1983	Mosquito	Burkina Faso	W	$10^{5.8}$
ArD38661SEN83[b]	1983	Mosquito	Senegal	W	$10^{7.3}$
R1662CAR85[b]	1985	Human	Central African Republic	C	$10^{6.5}$
SPU384001KEN97	1997	Human	Kenya	C	$10^{7.3}$
Ar2199SA00	2000	Mosquito	Saudi Arabia	C	$10^{7.0}$
SPU22.118KEN07[b]	2007	Human	Kenya	C	$10^{6.8}$

[a] C, southern, eastern, and central African lineage; E, Egyptian lineage; W, West African lineage.
[b] Strain selected for determination of analytical detection limit.

Communicable Diseases, Sandringham, South Africa. All the viruses were amplified either in Vero cells (CCL-81) or in suckling mice by using standard procedures (35).

Clinical specimens. Serial bleed samples ($n = 20$) taken from two sheep from day 0 to day 12 after experimental infection with the SPU22/07 Kenyan 2007 isolate of RVFV were tested; these samples included serum derived from clotted blood and plasma ($n = 6$) collected in EDTA as well as heparin blood collection tubes. In addition, routine diagnostic submissions received by the Special Pathogens Unit of the National Institute for Communicable Diseases in 2006 to 2008 from suspected cases of RVF in humans ($n = 65$) and animals ($n = 3$) in East and southern Africa were used; these included serum, liver, and kidney tissue specimens, as specified in Table 4.

Virus titration and isolation. Virus titration of infected tissue culture supernatants and sera from experimentally infected sheep was performed as described previously (31) with minor modifications. Briefly, sera were diluted tenfold in Eagle's minimum essential medium (EMEM; BioWhittaker) containing 100 IU penicillin, 100 μg streptomycin, and 0.25 μg amphotericin B (BioWhittaker). Four replicates of 100 μl per each dilution (from 10^{-1} to 10^{-7}) were transferred into flat-bottom 96-well cell culture microplates (Nunc), and equal volumes of Vero cell suspension in EMEM containing 2×10^5 cells/ml, 8% fetal bovine serum/ml (Gibco), and antibiotics were added. Inoculated microplates were incubated at 37°C in a CO_2 incubator and observed microscopically for cytopathic effects for 10 days postinoculation. Virus titers, calculated by the Kärber method (8), were expressed as $TCID_{50}$ per milliliter of sample. The limit of detection was 0.75 $\log_{10}$ $TCID_{50}$ per volume of sample tested. Isolations of RVFV from clinical specimens were done y inoculation of either 48-h monolayers of Vero cells or suckling mice by using standard laboratory procedures (35).

RNA extraction. Animal tissues were homogenized as 10% (wt/vol) suspensions in EMEM supplemented with antibiotics as described above. After centrifugation at $3,000 \times g$, supernatants free of cells were harvested and stored at -70°C. Genomic viral RNA was extracted from 140 μl of infected Vero cells tissue culture supernatants, liver and kidney tissue supernatants, or sera by using a QIAamp viral RNA mini kit (Qiagen, Germany) in accordance with the manufacturer's protocol. The extracted RNA was eluted in a total volume of 60 μl of buffer AVE and stored at -70°C until use.

TaqMan RTD-PCR. The TaqMan-RTD PCR assay was performed using a LightCycler RNA amplification kit HybProbe (Roche Diagnostics, Germany) and a Roche LightCycler instrument. Amplifications were carried out in 20-μl reaction mixtures containing 5 μl of the target virus RNA or the in vitro-transcribed RNA standard, 1 μM concentrations of each of the sense and antisense primers, and 5 mM $MgCl_2$. Cycling profiles, primers, and a TaqMan probe targeting a region of the G2 glycoprotein as described by Drosten et al. (2) were used.

RT-LAMP. RT-LAMP was carried out in a final reaction volume of 25 μl using a Loopamp RNA amplification kit (Eiken Chemical Co., Ltd. Tokyo, Japan) with 5 pmol (each) of the primers F3 and B3, 20 pmol (each) of the primers LF and LB, and 40 pmol (each) of the primers FIP and BIP. Five microliters of the extracted RNA was used as template per reaction. For real-time monitoring, the RT-LAMP reactions were incubated at 61°C for 60 min with a LA-200 Loopamp Realtime Turbidimeter (Teramecs, Japan) and inactivated at 80°C for 5 min. Positive and negative controls were included in each run of the assay.

Analysis of RT-LAMP product. (i) Real-time monitoring. A real-time turbidimeter was used to spectrophotometrically monitor every 6 s the accumulation of magnesium pyrophosphate at 400 nm. The cutoff values for positive samples were determined over time, when turbidity increased above the threshold value, which was fixed at 0.1. Results were analyzed using the LA-200E software package (Teramecs, Japan).

(ii) Agarose gel analysis. Five microliters of the RT-LAMP products were electrophoresed on a 2% molecular-grade agarose gel prepared in 0.5 × Trisborate-EDTA buffer stained with 0.5 μg/ml ethidium bromide. The amplification products were visualized with UV light at 302 nm.

(iii) Visualization with the naked eye. Reaction tubes were pulse centrifuged to deposit the magnesium pyrophosphate in the bottom of the tubes to detect amplification with the naked eye. Alternatively, 1 μl of fluorescent detection reagent (FDR; Eiken Chemical Co., Ltd., Tokyo, Japan) was added to the LAMP reaction. The FDR contains calcein complexed with manganese ions. Fluorescence occurs when pyrophosphate ions (formed as a by-product during LAMP amplification) removes the manganese ions from the calcein and binds to the calcein in the FDR. Fluorescence is further intensified when the calcein binds to magnesium ions in the reaction (16). For a positive reaction, orange changes to yellow fluorescence that can be detected with the naked eye or to green fluorescence under UV irradiation, while a negative reaction remains the orange

color of the unbound dye. The color change can be observed by the naked eye or with the aid of UV light at 302 nm, and the results are captured photographically.

(iv) Restriction endonuclease digestion. Amplification products were digested with BstXI (Roche, Germany) as suggested by the manufacturer and analyzed with a 2% agarose gel as described above.

In vitro transcription and quantification. To obtain a quantitative RNA standard, the diagnostic target region was amplified by standard reverse transcriptase PCR using RVFV RNA prepared from infected tissue culture supernatant and transcribed in vitro. The primers for the RVF RTD-PCR or RVF-LAMP outer primers were used, respectively, to generate amplicons representative of the RTD-PCR or LAMP targets. The target region PCR products were cloned into T7/Sp6 polymerase expression vector pCRII-TOPO (Invitrogen). The inserts were then amplified with vector-specific, universal M13 primers using standard PCR. The PCR products were purified using a Wizard SV Gel and PCR Clean Up system (Promega) and then in vitro transcribed and DNase digested using a MegaScript Sp6 kit (Ambion) according to the manufacturer's protocol. The RNA was purified according to the instructions of the RNeasy Protect kit (Qiagen, Germany) and quantified spectrophotometrically. The target RNA copy number was calculated, and serial dilutions ranging from 10^0 to 10^6 RNA copies were used to determine the range of quantification.

Analytical sensitivities of TaqMan RTD-PCR and RT-LAMP. To compare the analytical sensitivities of RT-LAMP and TaqMan RTD-PCR in the detection of decreasing number of RNA copies, tenfold dilution series of the RNA standard, ranging from 10^0 to 10^6 per reaction, were tested in six separate runs of each assay.

RESULTS

Optimum specific amplification was achieved at 61°C for 60 min. This was determined during the optimization of the RVF RT-LAMP when 10 pg of RNA (extracted from SPU 22/07) was incubated at a range of 61 to 65°C and could be detected only at 61°C. No nonspecific amplification was detected for the negative serum and tissue culture controls. A Tp value (time of positivity, i.e., time [in minutes] at which the turbidity increases above the threshold value fixed at 0.1) of $\leq$45 min and turbidity above the threshold value of $\geq$0.1 were considered a positive result (19). Real-time monitoring of turbidity allowed for the detection of amplification products as early as 16 min after initiation of the reaction, with the majority of positive specimens detected in less than 30 min.

Analytical sensitivity and specificity of RT-LAMP. When the sensitivity of the RT-LAMP was compared with that of a TaqMan RTD-PCR by testing tenfold serial dilutions of RNA prepared from infective tissue culture supernatant containing $10^{6.8}$ $TCID_{50}$/ml of the AR20368 RSA 81 isolate of RVFV, the two assays were equally sensitive, with a detection limit of 0.065 $TCID_{50}$ units per reaction volume (Fig. 1 A and B). The RT-LAMP detection limit of 0.065 $TCID_{50}$ units per reaction volume was further confirmed by testing tenfold serial dilutions of four additional strains of RVFV (Table 2), representing a phylogenetic spectrum of the virus (results not shown). High levels of analytical sensitivity for both assays were also demonstrated by measuring decreasing numbers of RNA copies. The RNA standard dose-response curves for TaqMan RTD-PCR and RT-LAMP were very similar to one another (R^2 of 0.8386 and 0.8042, respectively) and had the expected characteristic slope (Fig. 2). The borderline analytical detection limit as measured by number of RNA copies per reaction was 10 copies for both assays. During six runs, TaqMan RTD-PCR detected 10 RNA copies/reaction on four occasions (66.7%) and the RT-LAMP on three occasions (50%); however, this difference was not statistically significant ($P = 1.0000$). Both assays had 100% sensitivity in detecting $\geq$100
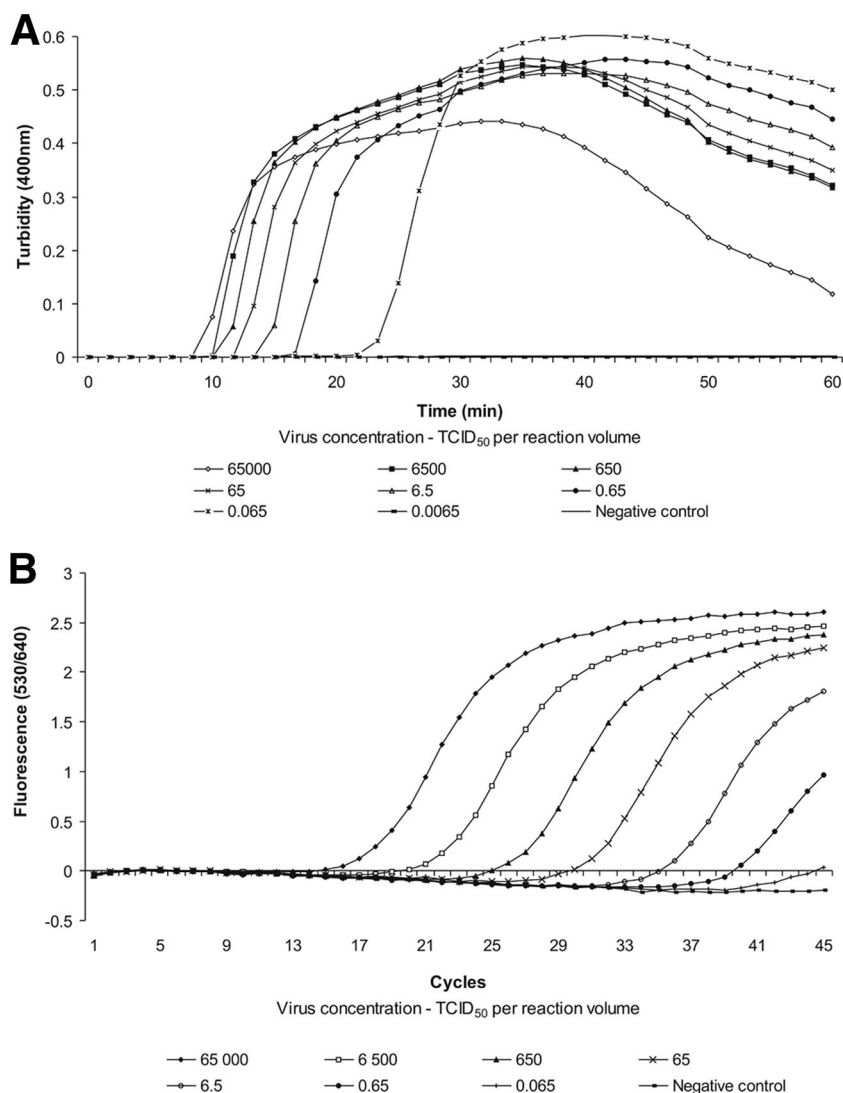
FIG. 1. Real-time kinetics of RT-LAMP (A) and TaqMan RTD-PCR (B) in tenfold serial dilutions of RNA prepared from infective tissue culture supernatant containing $10^{6.8}$ TCID$_{50}$/ml of the AR20368 RSA 81 isolate of RVFV.

RNA copies/reaction (Fig. 2). Despite distinct geographic and historic origins, all the RVFV isolates tested in this study were easily detectable by the RT-LAMP (Table 2), further confirming its high level of analytical sensitivity. The high level of analytical specificity of the assay was confirmed by the absence of amplification products when using the RVFV-specific L primer set with RNA extracted from highly concentrated stocks of six African phleboviruses related to RVFV and six other, unrelated arboviruses (results not shown). In addition, the specificity of the RT-LAMP amplification product was confirmed by restriction endonuclease digestion with BstXI, resulting in a product with the expected size of 209 bp (result not shown).

Alternative detection methods included agarose gel electrophoresis of the RT-LAMP products, which displayed the typical ladder-like pattern (Fig. 3), as well as FDR detection of amplification (Fig. 4). The fluorescent dye became yellow in positive samples (Fig. 4A) and showed deep green fluorescence with UV irradiation (Fig. 4B).

Diagnostic evaluation of RT-LAMP. Results of monitoring viremia by RT-LAMP, TaqMan RTD-PCR, and virus titration in two sheep experimentally infected with the wild-type isolate of RVFV are shown in Table 3. All three assays yielded negative results in sera collected before challenge. Of the sera collected on a daily basis postinfection, 11 tested positive and 9 tested negative in all tests (Table 3). The two molecular assays yielded the same results in experimental sheep plasma collected either in EDTA or heparin, but there was a slight delay in the detection of RNA extracted from heparin treated blood by the RT-LAMP.

Of the total of 32 human serum specimens which tested positive by virus isolation, all but one were also positive by the RT-LAMP and TaqMan RTD-PCR. One human serum sample which tested negative by virus isolation yielded positive results in the two molecular assays (Table 4). There was 100% agreement between the RT-LAMP, TaqMan RTD-PCR, and virus isolation results in detecting RVFV in liver and kidney tissues collected from three aborted buffalo fetuses (Table 4).
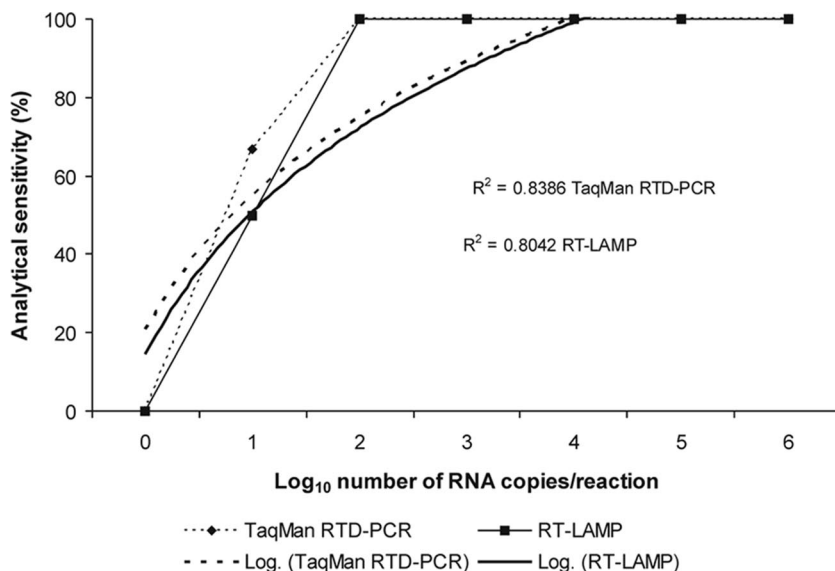
FIG. 2. Analytical sensitivity of RT-LAMP and TaqMan RTD-PCR as measured by the detection of numbers of RNA copies. Data were derived from testing tenfold dilution series of RNA standard during six runs of both assays. Logarithmic trend lines are added and correlation coefficients are calculated to determine the strength of linear data sets.

DISCUSSION

RVF is an important zoonotic disease and poses a potential bioterrorism threat (11, 26). The recent occurrence of the first confirmed outbreaks of RVF among humans and animals outside Africa (7), the ability of RVFV to replicate in a wide range of mosquito vectors (34), and the effects of global climate change which facilitate spread of arthropod-borne viruses (27) into regions of the world in which the virus is not endemic are of great medical and veterinary concern. The potential for further spread of RVFV outside its traditional geographic boundaries has resulted in increased international demand for validated molecular tools for the rapid diagnosis of RVF. RVFV belongs to the group of RNA viral hemorrhagic fever (VHF) agents, including Ebola virus, Marburg virus, Lassa and
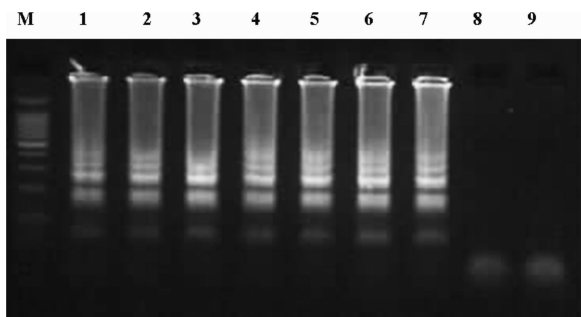

FIG. 3. Agarose gel electrophoresis profile of RT-LAMP products in serially diluted RNA of RVFV. Lane M, 100-bp molecular weight marker. Lanes 1 to 8, RT-LAMP products yielded from tenfold serial dilutions of RNA prepared from infective tissue culture supernatant containing $10^{6.8}$ $TCID_{50}$/ml of the AR20368 RSA 81 isolate of RVFV. Lanes 1 to 7, from left to right, 65,000 $TCID_{50}$ to 0.065 $TCID_{50}$/reaction volumes. Lane 8, 0.0065 $TCID_{50}$/reaction volume; not detected. Lane 9, negative control.
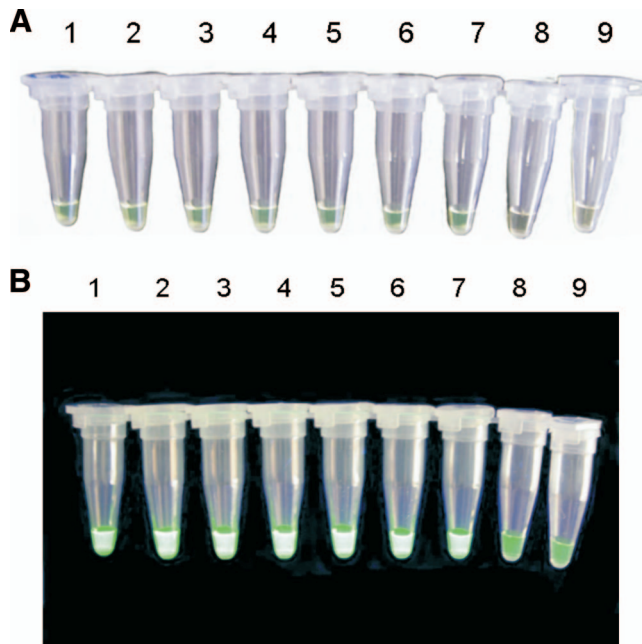

FIG. 4. Visual detection of RT-LAMP amplification products in tenfold dilutions of RVFV RNA. Tube 1 to 8 from left to right, RT-LAMP products yielded from tenfold serial dilutions of RNA prepared from infective tissue culture supernatant containing $10^{6.8}$ $TCID_{50}$/ml of the AR20368 RSA 81 isolate of RVFV; tube 9, negative control. Detection of the white precipitate (magnesium pyrophosphate) at the bottom of the tubes indicates a positive reaction, while the absence of precipitate indicates a negative reaction. After the addition of FDR to the reaction mix, fluorescence is detectable by the naked eye as a yellow color, while an orange color indicates a negative reaction (A); under UV irradiation, a positive reaction is indicated by a fluorescent bright green color (B).

TABLE 3. Monitoring of viremia in sheep experimentally infected with wild-type RVFV by RT-LAMP, TaqMan RTD-PCR, and virus titration

Experimental animal[a]	Day(s) after challenge	Result of:		
		RT-LAMP[b]	TaqMan RTD-PCR[b]	Virus titer[c]
Sheep 1	0	−	−	
	1	+	+	4.75
	2	+	+	7.75
	3	+	+	7.0
	4	+	+	5.5
	5	+	+	4.5
	6	+	+	2.5
	9, 12	−	−	
Sheep 2	0	−	−	
	1	+	+	3.65
	2	+	+	6.25
	3	+	+	4.75
	4	+	+	1.0
	5–10	−	−	

[a] Sheep experimentally infected with the SPU22/07 Kenyan 2007 isolate of RVFV.
[b] −, negative test result; +, positive test result.
[c] Log_{10} $TCID_{50}$/ml of serum.

TABLE 4. Comparison of RT-LAMP, TaqMan RT-PCR, and virus isolation results for the detection of RVFV in various clinical specimens

Type of specimen	Source tested	No. of samples	No. of specimens with indicated RT-LAMP, RTD-PCR, and virus isolation results			
			+, +, +[a]	−, −, −[b]	+, +, −[c]	−, −, +[d]
Serum[e]	Sheep	20	10	10	0	0
Plasma[e]	Sheep	6	6	0	0	0
Serum[f]	Human	65	31	32	1	1
Liver[f]	Buffalo[g]	3	3	0	0	0
Kidney[f]	Buffalo[g]	3	3	0	0	0
Total		97	53	42	1	1

[a] No. of specimens that tested positive (+) in all tests.
[b] No. of specimens that tested negative (−) in all tests.
[c] No. of specimens that tested positive by RT-LAMP and RTD-PCR but negative for virus isolation.
[d] No. of specimens that tested negative by RT-LAMP and RTD-PCR but positive for virus isolation.
[e] Specimens taken from sheep experimentally infected with wild-type RVFV.
[f] Specimens collected from suspected human and animal RVF cases during the disease outbreaks in Africa in 2006 to 2008.
[g] Aborted fetuses.

other arenaviruses, Crimean-Congo hemorrhagic fever virus, yellow fever virus, dengue virus, and hantaviruses. In the absence of hemorrhagic or specific organ manifestations, infections by VHF viruses are clinically difficult to recognize, with the implication that definitive diagnosis depends mainly on reliable laboratory tests (2). Although the range of possible VHF agents can be narrowed down by the patient's travel and exposure history, a suspected case and the causative virus must be rapidly identified to initiate specific or supportive treatment and to implement appropriate case management, infection control, and tracing of contacts.

An array of molecular techniques for rapid detection and identification of RVFV have been published, but data on their routine diagnostic performance are not available (2, 3). In this study, the utility of the LAMP technique for rapid and accurate detection of RVFV RNA in clinical specimens was investigated. Including the time required for the extraction of nucleic acid, detection of RNA in different clinical specimens of human and animal origin could be achieved within 2 h after arrival of the samples in the laboratory. Apart from the high levels of analytical and diagnostic accuracy and speed of detection, another important practical advantage of the LAMP technique is that it utilizes simple and relatively inexpensive equipment, which renders it promising for use in resource-poor settings. In addition, only basic molecular and technical skills are required for execution of the assay procedure, and interpretation of the results may be as simple as a visual evaluation of color change in the reaction mix. However, primer design for LAMP is more complex than for the conventional PCR-based assays, and specialized training and software are required for their design. Moreover, the development of LAMP requires the use of a set of multiple primers spanning a highly conserved 300-bp genomic region. For RVFV, this is easily achievable because there is no evidence of serolog-

ical subgroups or major antigenic variation between isolates of disparate chronologic or geographic origins (30), which is due to their low level of genetic diversity, irrespective of the genome segments analyzed (1). In the present work, we demonstrate that the set of LAMP primers targeting the RNA-dependent RNA-polymerase gene of the L segment of RVFV is well designed to detect all strains of the virus.

Results of the study show that the sensitivities of the RT-LAMP and TaqMan RTD-PCR assays are similar and that there is no cross-reactivity of the primers with the genes of related and unrelated arboviruses. Both assays had high levels of analytical sensitivity as measured by the detection of a known number of virus infectious doses and RNA copies of the in vitro-transcribed RNA standard. Similar analytical sensitivity for the TaqMan RTD-PCR based on the Superscript reverse transcriptase-Platinum *Taq* polymerase enzyme mixture was reported by Drosten et al. (2). By direct comparison of the two nucleic acid procedures with virus isolation results for clinical specimens from animals and humans, the diagnostic performance of the Taq-Man RTD-PCR assay for the detection of RVFV is also reported here for the first time. Specific nucleic acid targets in all positive specimens could be detected in this study in less than 45 min, with the majority detected in less than 30 min. This result confirms that the assay allows for rapid confirmation of clinical cases and early recognition of outbreaks. Visualization of amplification products with the naked eye, fluorescence, or agarose gel electrophoresis may be appropriate for most laboratory settings, while real-time monitoring of the accumulation of magnesium pyrophosphate in the reaction mix potentiates quantification of the assay. One has to emphasize, however, that definitive diagnosis or exclusion of RVF, as for any other suspected case of VHF, should not rely on a single PCR result. The LAMP should be run in parallel with additional tests, including the detection of type-specific antibodies to RVFV. In this con-

text, it is important to note that viremia in RVFV-infected individuals is of very short duration (30) and most infected humans and adult ruminants undergo subclinical or mild infections, but immunoglobulin M and immunoglobulin G antibodies are easily demonstrable shortly after exposure to the virus (22, 23).

ADDENDUM IN PROOF

We notice and acknowledge that an article describing a similar technique for the detection of Rift Valley fever virus (C. N. Peyrefitte, L. Boubis, D. Coudrier, M. Bouloy, M. Grandadam, H. J. Tolou, and S. Plumet, J. Clin. Microbiol. 46:3653–3659, 2008) was recently published in the Journal of Clinical Microbiology.

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