FULL PAPER *Parasitology*

Stability of Loop-Mediated Isothermal Amplification (LAMP) Reagents and its Amplification Efficiency on Crude Trypanosome DNA Templates

Oriel M. M. THEKISOE[1)], Raoul S. B. BAZIE[1)], Andrea M. CORONEL-SERVIAN[1)], Chihiro SUGIMOTO[2)], Shin-ichiro KAWAZU[1)] and Noboru INOUE[1)]*

[1)]*National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080–8555 and* [2)]*Research Center for Zoonosis Control, Hokkaido University, Sapporo, Hokkaido 060–0818, Japan*

(Received 17 June 2008/Accepted 1 December 2008)

ABSTRACT. This study evaluated the stability of LAMP reagents when stored at 25°C and 37°C, and also assessed its detection efficiency on different DNA template preparations. Accordingly, LAMP using reagents stored at 25°C and 37°C amplified DNA of *in vitro* cultured *T. b. brucei* (GUTat 3.1) from day 1 to day 15 of reagent storage. There were no significant differences (P>0.05) in detection sensitivity of LAMP among the reagents stored at 25°C, 37°C and –20°C (recommended storage temperature). LAMP using the reagents stored at above-mentioned temperatures amplified serially diluted DNAs (genomic DNA extracted by phenol-chloroform method, FTA card and hemolysed blood) of *T. b. gambiense* (IL2343) with high sensitivity. Reactions were conducted on the reagents stored from 1 day to 30 days. LAMP detection sensitivity was poor when fresh blood as DNA template was added directly into reactive solution. Results of this study demonstrated that LAMP has the potential to be used in field conditions for diagnosis of trypanosome infections without being affected by ambient temperatures of tropical and sub-tropical countries where trypanosomosis is endemic.
KEY WORDS: diagnosis, DNA template, LAMP, parasitic disease, *Trypanosoma*.

J. Vet. Med. Sci. 71(4): 471–475, 2009

Loop-mediated isothermal amplification (LAMP) is a method that amplifies DNA with high specificity, sensitivity and rapidity under isothermal conditions. LAMP employs a set of 2 specially designed inner (FIP and BIP) and outer (F3 and B3) primers [23]. Two more primers referred to LoopF and LoopB can be included in the reaction as they increase the sensitivity and acceleration of the reaction, then can reduce the reaction time by half [18]. The main advantage of LAMP over PCR is that it does not require complicated thermalcyclers and that amplification can be conducted within 1 hr in a laboratory heat block or water bath. LAMP results can be visualized by agarose gel electrophoresis with addition of fluorescent dyes under UV light [5, 12, 15, 17, 28], or by turbidity that can be seen by naked eye [4, 16, 27]. *Bst* DNA polymerase large fragment is utilized in LAMP reaction with optimal temperatures ranging from 60–65°C, and it can be inactivated at 80°C after the LAMP reaction [23]. LAMP has been widely developed for detection of various pathogens including viral, bacterial and protozoan diseases as well as application in embryo sexing [1, 6, 7, 9 14, 24, 29, 30]. With the above-mentioned advantages of LAMP, most of the literatures on development of this method for diagnoses of various infections conclude that this method is a strong candidate for application of molecular diagnoses particularly in the places where the use of PCR is prohibited due to the cost and environmental constraints [1, 10, 11, 14, 21, 23]. Moreover, *Bst* DNA polymerase appears not to be affected by known inhibitors of PCR found in blood components [2, 3, 8, 14].

There is, therefore, need to be conducted research that can determine the practicability of LAMP method being applied for diagnosis in the field, such as conducting LAMP experiments at field ambient temperatures, and determining simple DNA template preparations which could also be easily applied in the field. This study evaluates the stability of LAMP reagents when stored at 25°C and 37°C in comparison to –20°C (recommended storage temperature) for amplification of trypanosome DNA. Furthermore, the current study also assesses detection performance of LAMP on different DNA template preparations including fresh and hemolysed blood, filter paper and genomic DNA extracted by standard phenol-chloroform method. Trypanosome DNA is used as a target molecule in this study because our group has been developing LAMP for detection of animal and human trypanosome parasites with economic importance [14, 29, 30].

MATERIALS AND METHODS

Experimental set-up: The first set of experimental reactions was conducted over a period of 15 days on genomic DNA (gDNA) of *Trypanosoma brucei brucei* (GUTat 3.1 strain) whereby a master mix of the reagents for LAMP containing LAMP buffer with dNTPs, 6 LAMP primers, distilled water (DW) and target DNA together with a separate aliquot of *Bst* DNA polymerase (New England Biolabs Japan Inc., Tokyo) was stored in 25°C and 37°C dry incubators. LAMP using the reagents stored at –20°C (recommended storage temperature) was also conducted as control experiment run concurrently with the experimental reactions. Reactions were conducted by means of a real-time

* CORRESPONDENCE TO: Assoc. Prof. INOUE, N., National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080–8555, Japan.
e-mail: ircpmi@obihiro.ac.jp

LAMP turbidimetry (LA200, Teramecs, Tokyo) whereby threshold time for positive reaction was recorded and the data obtained were analysed by one-way ANOVA using S-plus 6 software for Windows (Insightful Corporation, Seattle, Washington, U.S.A.).

The second set of experiments was aimed at determining the detection performance of LAMP with the reagents stored at 25°C and 37°C in comparison to those stored at recommended storage temperature (–20°C). Reactions were examined on the LAMP reagents stored for 5 to 30 days with every 5 days. The *in vitro* cultured *T. b. gambiense* (IL2343) parasites were purified by anion exchange column (DE 52, Whatman, UK) and collected in PSG (NaH_2PO_4 $2H_2O$, Na_2HPO_4 $12H_2O$, NaCl and glucose) buffer. Parasites were counted and adjusted to 1×10^4/m*l*, then spun down and lysed. Un-infected cattle blood was spiked with the parasite lysates, and the blood was serially diluted 10 times as the concentration of lysate became to be corresponded to 1×10^4/m*l* down to 1×10^{-1}/m*l* parasites (1,000 *p*g down to 0.01 *p*g as DNA weight). Then, dilutions were used to prepare different DNA templates, that is, fresh and hemolysed blood, filter paper (FTA card®; Whatman, UK) and the genomic DNA of spiked blood extracted by phenol-chloroform method.

Genomic DNA (gDNA): The gDNA was extracted with a standard phenol-chloroform-isoamyl alcohol (PCI) method [26] from the cattle blood spiked with *T. b. gambiense* lysates corresponded to parasitemias of 1×10^4/m*l*, 1×10^3/m*l*, 1×10^2/m*l*, 1×10^1/m*l*, 1×10^0/m*l* and 1×10^{-1}/m*l*. Briefly, extraction buffer containing 10 mM Tris-HCl [pH 8.0], 10 mM EDTA, 1% sodium dodecyl sulphate and 100 μg/m*l* proteinase K was added to the samples and incubated overnight at 55°C. DNA was extracted with phenol-chloroform-isoamyl alcohol (25:24:1) and precipitated with isopropanol (PCI method). The pellet obtained was dissolved in 250 μl of DW, and each 1 μl of gDNA template was used for reactions.

Fresh and hemolysed blood as DNA templates: One microlitre of fresh cattle blood spiked with *T. b. gambiense* lysates corresponded to parasitemias of 1×10^4/m*l*, 1×10^3/m*l*, 1×10^2/m*l*, 1×10^1/m*l*, 1×10^0/m*l* and 1×10^{-1}/m*l* was poured into the reaction tube-containing LAMP master mix. For hemolysed blood, 10 μl of the fresh trypanosome spiked blood was mixed with 1 m*l* DW and spun at 1,500 × g for 10 min. After discarding supernatant, the precipitate was suspended in 20 μl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Then, 2 μl of hemolysed blood was added as DNA template for LAMP reactions.

Filter paper DNA: Cattle blood spiked with *T. b. gambiense* lysates corresponded to parasitemia of 1×10^4/m*l*, 1×10^3/m*l*, 1×10^2/m*l*, 1×10^1/m*l*, 1×10^0/m*l* and 1×10^{-1}/m*l*, was blotted on filter papers (FTA card®; Whatman, UK) and left to dry at room temperature for 1 hr. The filter papers were purified according to manufacturer's instructions. Briefly, 2 mm discs were cut out by a hole puncher (2.0 mm Harris Micro Punch; Whatman, UK) from the respective blood blotted filter papers and washed 3 times with 200 μl of FTA purification reagent (Whatman, UK), and twice with 200 μl of TE buffer. Thereafter, they were left to dry at 50°C for 10 min in a heat block (Dry Thermounit DTU 1B, TAITEC Co, Saitama, Japan) and then used as DNA templates.

LAMP: Table 1 shows a LAMP primer set used in this study for amplification of *T. b. brucei* PFR A gene reported by Kuboki *et al.* [14], though the loopF and loopB PFR A primers were designed in the current study. One microlitre of *T. b. brucei* DNA was added to a master mix (volume 24 μl) containing 12.5 μl LAMP buffer (40 mM Tris-HCl (pH 8.8), 20 mM KCl, 16 mM $MgSO_4$, 20 mM $(NH_4)_2SO_4$, 0.2% Tween 20, 1.6 M Betaine, 2.8 mM of each dNTP), 2.6 μl primer mix (50 μM stock of each FIP, BIP, F3, B3, LF and LB), 7.9 μl DW, and 1 μl *Bst* DNA polymerase. However, in the reactions in which 1 μl for fresh blood or 2 μl of hemolysed blood was used as template DNA, the DW volume was increased in order to adjust the total reaction volume. All LAMP reaction mixtures were incubated in a real-time LAMP turbidimeter (LA200, Teramecs, Tokyo) at 64°C for 30 min. LAMP products were electrophoresed in 1.5% Tris-acetic acid EDTA (TAE) agarose gel and stained with ethidium bromide solution for visualization.

RESULTS

LAMP reactions with the reagents stored at different temperatures: The *T. b. brucei* GUTat 3.1 DNA extracted from *in vitro* cultures and stored at –20°C was amplified by LAMP from day 1 to day 15 (terminated time of experiment) with the reagents stored at 25°C and 37°C for 15 days (Table 2). Threshold time for positive LAMP reactions ranged from 11'20" to 15'18" min, 11'19" to 13'33" min and 10'36" to 13'48" min for the LAMP reagents stored at 25°C, 37°C, and –20°C, respectively. These experiments were done in 3 repetitions. There were no significant differences ($P>0.05$) in the average threshold time between in the examined reagents stored at 25°C or 37°C and in the control reactions stored at –20°C.

Table 1. LAMP primer set used in this study

Parasite	Target gene	Accession #	Nucleotide sequences
T. b. brucei	PFR A	X14819	FIP: 5'TCAGAAGCGTCGAGCTGGGATTTTATCGACAATGCCATCGCC3' BIP: 5'CGCAAGTTCCTGTGGCTGCATTTTTTCCCAAGAAGAGCCGTCT3' F3: 5'TCACAACAAGACTCGCACG3' B3: 5'GGGCTTTGATCTGCTCCTC3' LF: 5'CAGTTCGTCTTCGATTTTCTCCAG3' LB 5'GATGAACGTGGCTGTTGTGC3'

Table 2. Threshold time for positive LAMP reactions for amplification of *T. b. brucei* DNA with the reagents stored at different temperatures

Temp.	25°C				37°C				–20°C			
	[a)]1st	[a)]2nd	[a)]3rd	Ave[b)]	1st	2nd	3rd	Ave	1st	2nd	3rd	Ave
D0[c)]	12'24"	12'20"	12'20"	12'21"	12'36"	12'24"	12'19"	12'26"	12'42"	12'18"	12'24"	12'28"
D1	12'12"	12'24"	12'24"	12'20"	12'12"	12'24"	12'24"	12'20"	11'48"	12'00"	12'24"	12'04"
D3	12'48"	13'24"	12'45"	12'59"	12'54"	12'44"	12'25"	12'41"	12'42"	12'13"	12'20"	12'25"
D6	12'36"	12'55"	12'17"	12'36"	12'48"	13'24"	12'50"	13'01"	13'36"	12'24"	12'24"	12'48"
D9	12'30"	12'24"	12'57"	12'37"	12'42"	13'33"	12'44"	13'00"	13'48"	12'00"	12'54"	12'54"
D12	15'18"	12'54"	14'34"	14'15"	12'48"	13'24"	11'19"	12'30"	12'06"	12'01"	12'45"	12'17"
D15	12'36"	11'20"	14'24"	12'47"	13'24"	12'30"	12'33"	12'49"	10'36"	12'56"	12'55"	12'09"

a) Reaction was done in three repetitions.
b) Average threshold time of the three repetitions.
c) Day 0 to day 15.

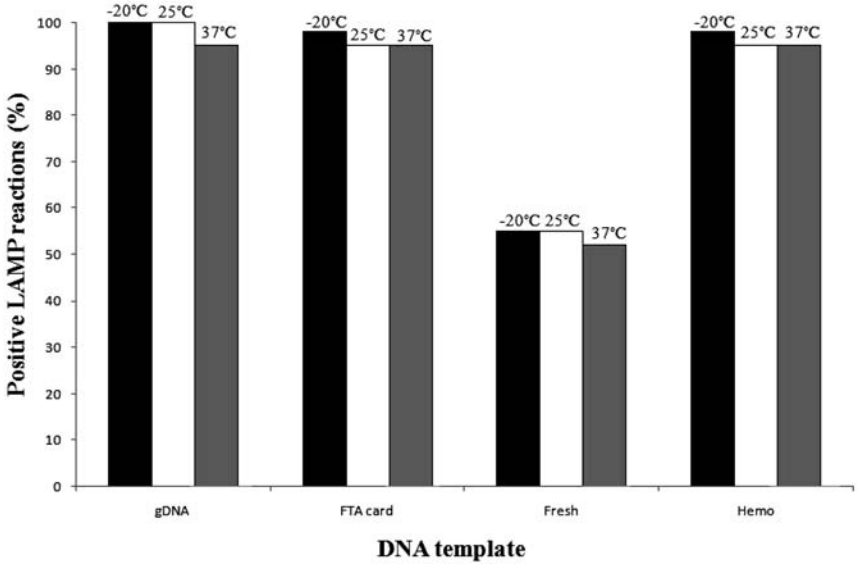

Fig. 1. Detection performance of LAMP method on different *T. b. gambiense* DNA template preparations with the reagents stored at –20°C, 25°C and 37°C. Average percentages for each DNA preparation were calculated from raw data shown in Table 3 for LAMP positive reactions of *T. b. gambiense* spiked blood with reactions conducted on 1 day, 5 days, 10 days, 15 days, 20 days, 25 days and 30 days of LAMP reagents storage. Fresh-fresh blood; Hemo-hemolysed blood; FTA card-blood dried on chemically treated filter paper; and gDNA-genomic DNA extracted by phenol-chloroform method.

LAMP detection performance on different T. b. gambiense DNA template preparations: Best detection performance (95–100%) was achieved when gDNA template was used, followed by FTA card and hemolysed blood templates. Storage temperatures of LAMP reagents did not affect the detection performance of LAMP (Fig. 1). This study further determined sensitivity of LAMP with the reagents stored at 25°C and 37°C on the serially diluted *T. b. gambiense* DNAs with concentrations of 1,000 *p*g, 100 *p*g, 10 *p*g, 1 *p*g, 0.1 *p*g and 0.01 *p*g. LAMP with the reagents stored at 25°C and 37°C amplified most of serially diluted DNA from 1,000 *p*g to 0.01 *p*g of gDNA, FTA card and hemolysed blood from day 1 to day 30 of reagent storage with high sensitivity, although poor detection sensitivity of LAMP with any reagents examined was observed when the fresh blood was used as DNA template (Table 3).

DISCUSSION

In the current study LAMP was performed with the reagents stored at 25°C and 37°C, which are possible ambient temperatures in tropical and sub-tropical countries where protozoan diseases such as trypanosomosis are endemic. There were no significant differences in detection performance of LAMP between the reagents stored at the above mentioned temperatures and those stored at –20°C, which was used as control in this study. The threshold time for positive LAMP reactions mostly did not differ among the LAMP reactions with the reagents stored at 25°C, 37°C and –20°C. Our results suggest that *Bst* DNA polymerase

Table 3. Detection sensitivity of LAMP with the reagents stored at –20°C, 25°C and 37°C on serially diluted *T. b. gambiense* DNA with different DNA templates

	1000pg	100pg	10pg	1pg	0.1pg	0.01pg
	Reactions with LAMP reagents stored at –20°C					
gDNA	100%*	100%	100%	100%	100%	100%
FTA card	100%	100%	100%	100%	100%	86%
Fresh blood	100%	71%	71%	29%	57%	0%
Hemolysed blood	100%	100%	100%	100%	100%	86%
	Reactions with LAMP reagents stored at 25°C					
gDNA	100%	100%	100%	100%	100%	100%
FTA card	100%	100%	100%	100%	86%	86%
Fresh blood	100%	71%	71%	14%	71%	0%
Hemolysed blood	100%	100%	100%	100%	86%	86%
	Reactions with LAMP reagents stored at 37°C					
gDNA	100%	100%	100%	100%	86%	86%
FTA card	100%	100%	100%	100%	86%	86%
Fresh blood	100%	43%	71%	29%	71%	0%
Hemolysed blood	100%	100%	100%	100%	71%	100%

* Percentages were calculated from a number of positive LAMP reaction from a total number of reactions conducted on each of day1, day5,day10, day15, day20, day 25 and day30 of LAMP reagents storage for detection of trypanosomes from each DNA concentration indicated above.

which is the critical reagent in gene amplification reaction can amplify DNA when it is even not kept at freezing temperatures.

In order to develop simplified procedures for DNA template preparation in LAMP, DNA templates prepared by different methods were examined. As a result, detection efficiency of LAMP was improved when gDNA template extracted by standard PCI method, FTA card and hemolysed blood were used. In these conditions, serially diluted DNA from 1,000 *p*g to 0.01 *p*g was amplified despite reagents preserved at different temperatures. The 0.01 *p*g trypanosome concentration is equivalent to less than 1 trypanosome cell [20]. However, LAMP detection performance was poor when fresh blood was used as DNA template. We suppose that as it was not easy to weaken the bonds of double stranded DNA in crude blood, the results in poor or no amplification was obtained.

The critical factor for accurate and reliable amplification seems to be the kind of DNA template. Fresh blood DNA template is easy to prepare, but it offers unspecified DNA which affects the specificity of gene amplification method. However, Poon *et al.* [25] have reported that the *Plasmodium* DNA detected by LAMP using a promising simple DNA template preparation method from heat-treated blood. Furthermore, Njiru *et al.* [21, 22] reported that LAMP can also amplify trypanosome DNA from heat treated blood, buffy coat and serum. Kuboki *et al.* [14] also reported that LAMP can amplify trypanosome DNA from FTA cards easier than PCR which requires special reagents to remove inhibitors in the filter paper DNA template [19].

Highly purified DNA template is desirable for precise gene amplification. However, the current standard procedures for gDNA extraction and purification are tedious and labour-intensive [3]. Filter paper DNA template preparation is relatively easy and rapid, but it offers partially purified DNA. In reactions using gDNA, positive LAMP results can be seen by turbidity in the reaction tube [16]. However it is not possible when using fresh and hemolysed blood as DNA templates, while when using filter paper DNA templates, it is possible but difficult to observe turbidity.

In conclusion, this study revealed that LAMP reagents are relatively stable when stored at 25°C and 37°C that are warmer temperatures than recommended storage temperatures by the manufacturers. This fact further emphasizes the possibility of application of LAMP in field conditions as previously reported [8, 10, 11, 13, 21–23]. However, further improvements on rapid and simpler DNA extraction and purification methods are required to enhance the practicability of applying LAMP method for diagnoses in the field.

ACKNOWLEDGEMENTS. The first author was supported by a research grant fellowship from the Japanese Society for the Promotion of Science (JSPS) for young scientists. This study was also supported by (i) Grant-in-Aid for Scientific Research from JSPS, (ii) Grant from Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN).

REFERENCES

1. Alhassan, A., Thekisoe, O. M. M., Yokoyama, N., Inoue, N., Motloang, M. Y., Mbati, P. A., Yin, H., Katayama, Y., Anzai, T., Sugimoto, C. and Igarashi, I. 2007. Development of Loop-mediated isothermal amplification (LAMP) method for diagnosis of equine piroplasmosis. *Vet. Parasitol.* **143**: 155–160.
2. Al-Soud, W. A., Jonsson, L. J. and Radstrom, P. 2000. Identification and characterization of immunoglobulin G in blood as a major inhibitor of diagnostic PCR. *J. Clin. Microbiol.* **38**: 345–

350.
3. Al-Soud, W. A. and Radstrom, P. 2001. Purification and characterization of PCR-inhibitory components in blood cells. *J. Clin. Microbiol.* 39: 485–493.
4. Cho, H. S. and Park, N. Y. 2005. Detection of canine distemper virus in blood samples by reverse transcription loop-mediated isothermal amplification. *J. Vet. Med.* **52**: 410–413.
5. Dukes, J. P., King, D. P. and Alexandersen, S. 2006. Novel reverse transcription loop-mediated isothermal amplification for rapid detection of foot and mouth disease virus. *Arch. Virol.* 151: 1093–1106.
6. Endo, S., Komori, T., Ricci, G., Sano, A., Yokoyama, K., Ohori, A., Kamei, K., Franco, M., Miyaji, M. and Nishimura, K. 2004. Detection of gp43 of *Paracoccidioides brasiliensis* by the loop-mediated isothermal amplification (LAMP) method. *FEMS Microbiol. Letters.* **234**: 93–97.
7. Enosawa, M., Kageyama, S., Sawai, K., Watanabe, K., Notomi, T., Onoe, S., Mori, Y. and Yokomizo, Y. 2003. Use of loop-mediated isothermal amplification of the IS900 sequence for rapid detection of cultured *Mycobacterium avium* subsp. *paratuberculosis*. *J. Clin. Microbiol.* **41**: 4359–4365.
8. Grab, D.J., Lonsdale-Eccles, J. and Inoue, N. 2005. LAMP for tadpoles. *Nature Methods* **2**: 635.
9. Hirayama, H., Kageyama, S., Moriyasu, S., Sawai, K., Onoe, S., Takahashi, Y., Katagiri, S., Toen, K., Watanabe, K., Notomi, T., Yamashina, H., Matsuzaki, S. and Minamihashi, A. 2004. Rapid sexing of bovine preimplantation embryos using loop-mediated isothermal amplification. *Theriogenology* **62**: 887–896.
10. Ihira, M., Yoshikawa, T., Enomoto, Y., Akimoto, S., Ohashi, M., Suga, S., Nishimura, N., Ozaki, T., Nishiyama, Y., Notomi, T., Ohta, Y. and Asano, Y. 2004. Rapid diagnosis of human herpesvirus 6 infection by a novel DNA amplification method, loop-mediated isothermal amplification. *J. Clin. Microbiol.* **42**: 140–145.
11. Ikadai, H., Tanaka, H., Shibahara, N., Matsuu, A., Uechi, N., Itoh, N., Oshiro, S., Kudo, N., Igarashi, I. and Oyamada, T. 2004. Molecular evidence of infections with *Babesia gibsoni* parasites in Japan and evaluation of the diagnostic potential of a loop-mediated isothermal amplification method. *J. Clin. Microbiol.* **42**: 2465–2469.
12. Iwamoto, T., Sonobe, T. and Hayashi, K. 2003. Loop-mediated isothermal amplification for direct detection of *Mycobacterium tuberculosis* complex, *M. avium*, and *M. intracellulare* in sputum samples. *J. Clin. Microbiol.* **41**: 2616–2622.
13. Kaneko, H., Kawana, T., Fukushima, E. and Suzutani, T. 2006. Tolerance of loop-mediated isothermal amplification to a culture medium and biological substances. *J. Biochem. Biophys. Methods.* **70**: 499–501.
14. Kuboki, N., Inoue, N., Sakurai, T., Di Cello, F., Grab, D. J., Suzuki, H., Sugimoto, C. and Igarashi, I. 2003. Loop-mediated isothermal amplification for detection of African trypanosomes. *J. Clin. Microbiol.* **41**: 5517–5524.
15. Maruyama, F., Kenzaka, T., Yamakuchi, N., Tani, K. and Nasu, M. 2003. Detection of bacteria carrying the *stx*^{2} gene by in situ loop-mediated isothermal amplification. *Applied Environ. Microbiol.* 69: 5023–5028.
16. Mori, Y., Nagamine, K., Tomita, N. and Notomi, T. 2001. Detection of loop-mediated isothermal reaction by turbidity derived from magnesium pyrophosphate formation. *Biochem. Biophys. Res. Commun.* **289**: 150–154.
17. Mori, Y., Hirano, T. and Notomi, T. 2006. Sequence specific visual detection of LAMP reactions by addition of cationic polymers. *BMC Biotech.* **6**: 1–10.
18. Nagamine, K., Hase, T. and Notomi, T. 2002. Accelerated reaction by loop-mediated isothermal amplification using loop primers. *Mol. Cell. Probes.* **16**: 223–229.
19. Nishimura, N., Nakayama, T., Tonoike, H., Kojima, K. and Kato, S. 2000. Direct polymerase chain reaction from whole blood without DNA isolation. *Ann. Clin. Biochem.* **37**: 674–680.
20. Njiru, Z. K., Ndungu, K., Matete, G., Ndungu, G. M., Gibson, W. C. 2004. Detection of *Trypanosoma brucei rhodesiense* in animals from sleeping sickness foci in East Africa using serum resistance (SRA) gene. *Acta Trop.* **90**: 249–254.
21. Njiru, Z. K., Mikosza, A. S., Matovu, E., Enyaru, J. C., Ouma, J. O., Kibona, S. N., Thompson, R. C. and Ndungu, J. M. 2007. African trypanosomiasis: Sensitive and rapid detection of subgenus *Trypanozoon* by loop-mediated isothermal amplification (LAMP) of parasite DNA. *Int. J. Parasitol.* **38**: 589–599.
22. Njiru, Z. K., Mikosza, A. S. J., Armstrong, T., Enyaru, J. C., Ndungu, J. M. and Thompson, A. R. C. 2008. Loop-mediated isothermal amplification (LAMP) method for rapid detection of *Trypanosoma brucei rhodesiense*. *PLoS Negl. Trop. Dis.* **2**: e147.
23. Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N. and Hase, T. 2000. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res.* **28**: e63.
24. Pham, H. M., Nakajima, C., Ohashi, K. and Onuma, M. 2005. Loop-mediated isothermal amplification for rapid detection of Newcastle disease virus. *J. Clin. Microbiol.* **43**: 1646–1650.
25. Poon, L. L. M., Wong, B. W. Y., Ma, E. H. T., Chan, K. H., Chow, L. M. C., Abeyewickreme, W., Tangpukdee, N., Yuen, K. W., Guan, Y., Looareesuwan, S. and Malik Peiris, J. S. 2006. Sensitive and inexpensive molecular test for *Falciparum* Malaria: Detecting *Plasmodium falciparum* DNA directly from heat-treated blood by loop-mediated isothermal amplification. *Clin. Chem.* **52**: 303–306.
26. Sambrook, J. and Russell, D. W. 2001. Preparation and analysis of eukaryotic genomic DNA. pp 6.1–6.30. *In*: Molecular Cloning (Sambrook J. and Russell D. W. eds.), Cold Spring Harbor Laboratory Press, New York.
27. Savan, R., Kono, T., Itami, T. and Sakai, M. 2005. Loop-mediated isothermal amplification: an emerging technology for detection of fish and shellfish pathogens. *J. Fish. Dis.* **28**: 573–581.
28. Soliman, H. and El-Matbouli, M. 2005. Reverse transcription loop-mediated isothermal amplification (RT-LAMP) for rapid detection of viral hemorrhagic septicaemia virus (VHS). *Vet. Microbiol.* **114**: 205–213.
29. Thekisoe, O. M. M., Inoue, N., Kuboki, N., Tuntasuvan, D., Bunnoy, W., Borisutsuwan, S., Igarashi, I. and Sugimoto, C. 2005. Evaluation of loop-mediated isothermal amplification (LAMP), PCR and parasitological tests for detection of *Trypanosoma evansi* in experimentally infected pigs. *Vet. Parasitol.* **130**: 327–330.
30. Thekisoe, O. M. M., Kuboki, N., Nambota, A., Fujisaki, K., Sugimoto, C., Igarashi, I., Yasuda, J. and Inoue, N. 2007. Species-specific loop-mediated isothermal amplification (LAMP) for diagnosis of trypanosomosis. *Acta Trop.* **102**: 182–189.