A Simple Method for the Detection of Measles Virus Genome by Loop-Mediated Isothermal Amplification (LAMP)

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Approximately 20,000–30,000 measles patients were reported in a surveillance of infectious diseases because of low vaccine coverage of 80% in Japan. Among them, some were thought to be secondary vaccine failure (SVF) with generally mild or non-typical measles illness and sometimes became a source of further transmission. We have developed a new, sensitive, and rapid method to detect the measles virus genome by reverse transcription loop-mediated isothermal amplification (RT-LAMP). We examined 50 nasopharyngeal secretion (NPS) samples that were obtained during the 1999 outbreak and stored at −70°C and fresh NPS, lymphocytes and sera from 11 patients in 2003. Total RNA was extracted from the samples and subjected to reverse transcription-polymerase chain reaction (RT-PCR) and RT-LAMP. We detected the genomic RNA corresponding to at least 0.01–0.04 TCID50, 30–100 copies in samples by RT-LAMP within 60 min after extraction of RNA, and all four genotypes isolated in Japan were equally amplified. Specific DNA amplification was monitored spectrophotometrically by real time turbidimeter and the quantity of RNA was calculated. Measles virus genome was detected in 44 of 50 stored NPS by RT-PCR and in 49 by RT-LAMP. The vaccine strain was discriminated from wild strains after sequencing the LAMP products. RT-LAMP is a useful rapid diagnostic method for the detection of measles virus without any special apparatus, showing higher sensitivity than RT-PCR, and expected to be applied for hospital-based infection control and for laboratory-based measles surveillance.

INTRODUCTION

Measles virus is a single stranded negative sense RNA virus, belonging to the genus Morbillivirus, family Paramyxoviridae, order Mononegavirales. It consists of 15,894 nucleotides, coding six structural proteins; nucleoprotein (N), phosphoprotein (P), membrane (M), fusion (F), hemagglutinin (H), and large (L) proteins [Griffin et al., 2001]. Two glycoproteins of F and H are components of outer surface envelope proteins and cooperatively play an important role in the initial attachment of virus to the cells and following virus-cell fusion [Wild et al., 1991; Griffin et al., 2001]. Genome RNA is surrounded by N, P, and L proteins and they comprise the ribonucleoprotein complex (RNP) [Griffin et al., 2001]. Measles virus is considered to have monotypic characteristics but genetic variation has been described for the wild types. The standardized nomenclature for describing the genetic characteristics of wild-type measles virus isolates is demonstrated using 8 clades, designated A, B, C, D, E, F, G, and H, with 22 genotypes [WHO, 2001]. The differentiation of measles virus genotypes is based on the sequence results of the most variable region of 500 nucleotides at the 3’ end of the N gene or full-length H gene. In our previous work, we have developed a sensitive and rapid method for the detection of measles virus by reverse transcription-loop-mediated isothermal amplification (RT-LAMP). This method has several advantages over conventional methods such as high sensitivity, rapidity, and simplicity.

KEY WORDS: measles virus; reverse transcription-polymerase reaction (RT-PCR); reverse transcription-loop-mediated isothermal amplification (RT-LAMP); rapid diagnosis
reports [Nakayama et al., 1995; Yamaguchi, 1997; Takahashi et al., 2000], the measles virus strains isolated in Japan since 1984 were classified into four different genotypes, C1 (before 1985), D3 (1985–1990), D5 (after 1990), Chicago-type D3 (1997–1999), and H1 genotype (after 2000) [Zhou et al., 2003].

Measles is still a major killer among infants worldwide and the total number of cases is estimated to be more than 30 million despite approximately 80% of vaccine coverage. Meanwhile, measles-related deaths are estimated to be 0.77 million [WHO, 2002; Stein et al., 2003]. Recently, several problems have come into existence, such as sporadic measles outbreaks, an increase in the number of secondary vaccine failure (SVF) and transmission in high school or among university students and adults [Helfand et al., 1998; WHO, 2002; Nakayama et al., 2003]. Some modified measles patients with SVF were not correctly diagnosed without any virological examinations because of their non-typical measles illnesses and caused further transmission among susceptible individuals.

The diagnosis of virus infection is traditionally performed by virus isolation and serological examinations, but these methods are time-consuming and not appropriate for clinical setting. In some virus laboratories, molecular-based diagnostic methods, such as reverse transcription-polymerase chain reaction (RT-PCR) and hybridization were employed. Recently, real time RT-PCR has been developed, showing its rapidity and quantitative features [Ozoemen et al., 2004; Schalk et al., 2004]. Approximately 100–280 copies of the measles virus genome were detected [Ozoemen et al., 2004; Schalk et al., 2004], but it is not appropriate as a rapid diagnostic tool for clinical use because it requires a specific apparatus. A more sensitive and specific method for DNA amplification method, loop-mediated isothermal amplification (LAMP), was developed by one of the authors [Notomi et al., 2000]. This method employed Bst DNA polymerase with strand displacement activity and a set of four specially designed primers that recognize a total of six different sequences in the target DNA. The key reaction is the construction of a 5' and 3' end loop dumb-bell structure and multi-branched stem-loop products are amplified through repetition of the reactions. The distinctive features of LAMP are rapidity, high sensitivity, high specificity, and simplicity; these are required for the rapid diagnosis. We developed this new method for the detection of the measles virus genome by real-time reverse transcription-loop-mediated isothermal amplification (RT-LAMP) targeting the measles N gene and compared its sensitivity with nested RT-PCR reported previously [Nakayama et al., 1995; Zhou et al., 2003].

MATERIALS AND METHODS

Measles Virus Strains

All measles virus strains used in this study were isolated in Japan from 1984 to 2002; genotype A [Edmonston, AIK-C], genotype C1 [MV/Tokyo.JPN/84-E], genotype D3 [MV/Tokyo.JPN/87-K], Chicago-type D3 [MV/Tokyo.JPN/37.99(Y)], genotype D5 [MV/Tokyo.JPN/2000-KA], and Genotype H1 [MV/Tokyo.JPN/20.00(S)]. They were isolated from nasopharyngeal swabs (NPS) or peripheral blood mononuclear cells (PBMC) by Vero cells before 1985 and B95a cell cultures after 1987 [Nakayama et al., 1995; Yamaguchi, 1997; Takahashi et al., 2000; Zhou et al., 2003]. To examine the sensitivity of RT-LAMP, we used 50 nasopharyngeal secretion (NPS) samples stored at −70°C obtained from natural measles and fresh samples were obtained from 11 patients; 8 were suspected as having SVF and 3 had vaccine-associated illness.

RT-PCR

Total RNA was extracted from 200 µl of virus culture fluid or clinical samples with a magnetic bead RNA extraction kit (TOYOBO Co. Ltd., Osaka, Japan), and the RNA pellet was suspended in 25 µl of distilled water. It was subjected to nested RT-PCR and RT-LAMP targeted at the COOH terminus of the N protein region known as the most variable region [WHO, 2001]. The measles virus genome was first converted to cDNA with N-430(+) primer (5'-ATTAGTAGTGATCAATCCAGG-3') with AMV reverse transcriptase (Life Technologies, Inc., Gaithesburg, MD). The first PCR was performed with a set of N-850(+) (5'-TAGAATACTGTATGCTG-3') and MPX(-) (5'-AGGCCTGATTGAACCATGAT-3'), and the nested PCR was done with N1200(+) (5'-GATCCAGATTTTATTAGATTG-3') and NP-P(2-) (5'-AGGTTAGGCGGATGTGTCTT-3'). PCR was performed using 1.25 U of Taq DNA polymerase (TaKaRa BioMedicals, Tokyo, Japan) by TaKaRa thermal cycler (TaKaRa BioMedicals) with 30 rounds of thermal cycling conditions; denature at 93°C for 1 min, reannealing at 58°C for 1 min, and extension at 72°C for 2.5 min. PCR products were confirmed by electrophoresis through 1.5% agarose gel stained with ethidium bromide, as previously reported [Nakayama et al., 1995; Yamaguchi, 1997; Zhou et al., 2003].

Measles Virus RT-LAMP

LAMP method was characterized by auto-cycling strand displacement DNA synthesis with Bst DNA polymerase (New England Biolabs, Beverly, MD) and a specially designed set of primers. The principle of primer design is shown in Figure 1A and the LAMP primer is targeted for the N region similar to the RT-PCR region from the genome position 1242 to 1442 (Fig. 1B). We synthesized six LAMP primers recognizing eight different regions, referred to the software program for LAMP primer design (Eiken Chemical Co. Ltd., Tokyo, Japan); two outer primers (F3 and B3), two inner primers, a forward inner primer (FIP) and a backward inner primer (BIP), and two loop primers (Loop F and Loop B), and primer sequences are shown in Figure 1C. The FIP contains the complementary alignment of F1 linked with the F2 sequence (F1C + F2), and BIP contains the complementary sequence of B1 sequence linked with the
B2 (B1C + B2). Basically, these four primers amplified the target DNA and we synthesized two additional loop primers F and B located between F1 and F2, and between B1 and B2, respectively. The addition of two loop primers enhances the specificity and reactivity [Nagamine et al., 2002]. For the LAMP reaction, the mixture was made up to a total of 25 µl of reaction mixture, containing 40 pmol (each) of FIP and BIP, 5 pmol (each) of F3 and B3, 20 pmol (each) of Loop F and Loop B, 1.4 mM each dNTPs, 0.8M betaine, 20 mM Tris-HCl, 10 mM KCl, 10 mM (NH4)2SO4, 8 mM MgSO4, 0.1% Tween 20, 1 U AMV reverse transcriptase (New England Biolabs), 8U Bst DNA polymerase (New England Biolabs), and 5 µl of sample RNA. The reaction mixture was subjected to real-time turbidimeter LA200 (TERAMECS, Kyoto, Japan) [Mori et al., 2004] and the LAMP reaction was carried out at 63°C for 60 min. The turbidity was scanned every 6 sec.

The diagram of LAMP is shown in Figure 2. Measles genome is a negative sense RNA and then first converted to cDNA with F2 portion of FIP primer in Figure 2(1) by AMV reverse transcriptase. F3 primer extends the cDNA synthesis with displacement of RNA-cDNA double strand in Figure 2(2). The reverse transcription process produces two kinds of structures; RNA-cDNA complex from F3 to B3 portion and single strand cDNA primed by FIP primer in Figure 2(3). This cDNA forms 5’ end loop structure. BIP primer anneals to 3’ end of cDNA and extends DNA synthesis in Figure 2(3)(4). B3 primer attaches the B3 portion and detaches the double strand DNA in Figure 2(5). Thereafter, double strand DNA and dumb-bell loop structure of single strand DNA are produced in Figure 2(6)(7). This dumb-bell loop structure is basic product for further extension of LAMP reaction and FIP primer binds to the 3’ end of single strand loop region in Figure 2(7). Similar DNA synthesis with displacement activity continues with cycling reaction and multi-branched loop structures are synthesized [Notomi et al., 2000].

As the LAMP reaction progresses, the reaction by-products pyrophosphate ions bind to magnesium ions and they form white precipitates of magnesium pyrophosphate. Light (650 nm) emitted by light emitting diodes passes through PCR tubes containing the LAMP solution and illuminates the photodiode on the opposite side. The turbidity is calculated based upon the ratio between the intensity of light received by photodiode and emitted light intensity. Thus, measurement of the
turbidity closely related to the amplification of DNA and the turbidity >0.1 was considered as LAMP positive [Mori et al., 2004].

Sequencing of the LAMP Products

The LAMP product was purified by a magnetic bead DNA purification kit (TOYOBO Co. Ltd.) and was sequenced with the F2 primer by dye terminator method using ABI 377A sequencer (Applied Biosystems, Foster city, CA).

Construction of the N Protein Expression Plasmid

We have already reported the construction of N protein expression plasmid for reverse genetics [Kumada et al., 2004]. Coding region of the N gene was cloned in pBleuscript SK II-vector at the downstream of T7 promoter. Plasmid was linearized by Spe I digestion and RNA was transcribed by T7 RiboMAX Express Large Scale RNA Production System (Promega, Madison, WI). The transcribed RNA was used as a template for RT-LAMP.

RESULTS

Sensitivity of LAMP

Mvi/Tokyo.JPN/87-K strain of genotype D3 was used to examine the sensitivity of the LAMP method and the results are shown in Figure 3. The virus contained $2 \times 10^3$ TCID 50/200 μl. RNA was extracted from 200 μl and suspended in 25 μl. The RNA was serially diluted by 1:10 and 5 μl was used for nested RT-PCR and RT-LAMP. The threshold for a positive reading of the spectrophotometric value was defined as 0.1 [Mori et al., 2004]. Measles virus genome was detected at a 10^-4 dilution by RT-LAMP and at 10^-3 dilution by nested RT-PCR. At least 0.04 TCID 50 genome was detected by RT-LAMP. We synthesized the measles N gene RNA and the sensitivity was examined. The detection limit was estimated as 30–100 copies of RNA in the sample (data not shown).

We compared the sensitivity of LAMP for different genotypes of measles virus and the results of MVi/Tokyo.JPN/2000-KA (genotype D5), MVi/Tokyo.JPN/...
37.99(Y)(genotype D3), and MVi/Tokyo.JPN/20.00(S)(genotype H1) are shown in Figure 4A. The infectivity of genotypes D5 was $6 \times 10^3$ TCID50/200 ml and RT-LAMP showed positive for $10^{-5}$ dilution. The detection limit was estimated as 0.012 TCID50. D3 and H1 contained $8 \times 10^4$ TCID50/200 ml, and $3 \times 10^4$ TCID50/200 ml, respectively. RT-LAMP was positive for $10^{-6}$ dilution of both genotype strains and detection limit was estimated as 0.016–0.006 TCID50 with similar sensitivity. All genotypes (A, C1, D3, D5, and H1) were equally amplified.

We analyzed the correlation between the time (in seconds) to reach the threshold $>0.1$ of turbidity and infectivity (TCID50). The result using MVi/Tokyo.JPN/20.00(S)(genotype H1) is shown in Figure 4B. A linear correlation was obtained: $y$ (TCID50) = $-0.0064 \times$ (seconds) + 10.123. Using the equation, we calculated the virus genome quantity related to the infectivity of the samples.

Multi-branched stem loop structure is the characteristics of LAMP and LAMP products demonstrated the typical ladder pattern. Specific amplification was confirmed that the ladder-like LAMP products became a single band after digestion with specific restriction enzyme. Eco47I site is demonstrated in Figure 1B. The results of electrophoresis are shown in Figure 4C. LAMP products of D5, D3, and H1 exhibited a ladder pattern in lanes 1, 3, and 5 and after digestion with Eco47I they became a single DNA band (lanes 2, 4, and 6).

**Detection Rate by RT-PCR and LAMP**

We used 50 NPS samples from the patients diagnosed clinically as having a measles infection in 1999 and they were stored at $-70$ °C for 4 years. The results of the RT-PCR and LAMP are shown in Table I. Measles virus was not isolated from the stored samples but the genome was detected in 49 by RT-LAMP and in 44 by nested RT-PCR. Fresh samples were obtained from 11 patients. Measles virus was isolated from two NPS samples and the measles genome was detected in 8 from 11 NPS by nested RT-PCR and in 9 by RT-LAMP. It was also detected in all PBMC and serum samples by RT-LAMP with higher sensitivity than by RT-PCR.

**Sequence Analysis of LAMP Products of Measles Infection**

Wild-type measles virus genotypes are now classified into 22 genotypes [WHO, 2001] and we depicted the sequence alignments of 22 reference strains in the target

![Figure 4](image-url)
region of the N gene (Fig. 5). Two hundred one nucleotides were amplified from genome position 1242 to 1442 by RT-LAMP.

After immunization with live measles vaccine, approximately 10% of the recipients developed febrile reactions and rash. We obtained NPS, PBMC, and sera from three patients with vaccine-associated illness. In three recipients, measles genome was detected from PBMC and plasma. LAMP products were purified and sequenced by F2 primer. They were identified as genotype A and the others were H1 wild genotype circulating in Japan.

**DISCUSSION**

In Japan we still have annual outbreaks and especially school outbreaks are reported in teenagers and adults [Nakayama et al., 2003]. They had a past history of immunization and did not show the typical measles illness [Helfand et al., 1998; Mossong et al., 1999, Lievano et al., 2004]. They were initially diagnosed as having toxic dermatitis, a drug allergy, or an unknown viral infection. When they were diagnosed as having measles infection by conventional serological examination, they extended the infection to persons who were in contact with them [Mossong et al., 1999]. In this standpoint of view, rapid virological diagnostic kits are expected for the diagnosis of the patients with vaccine-modified non-typical measles.

Virus isolation takes more than 1 week, even using sensitive B95a cells [Kobune et al., 1990]. As for the serological response, the detection of IgM EIA antibodies was employed in a clinical setting, but the negative for IgM EIA does not always imply negativity in terms of virus infection [Griffin and Bellini, 2001]. A recent development in the molecular approach was applied for a clinical diagnostic tool. We reported the use of nested RT-PCR for the detection of the measles virus genome, and it showed a high sensitivity and specificity.
[Nakayama et al., 1995; Afzal et al., 2003]. RT-PCR procedures established at various laboratories have differed in sensitivity by as much as 1,000-fold [Afzal et al., 2003]. Recently, several authors have reported real-time RT-PCR, which has the additional advantage of being a quantitative method, but this method was not appropriate for rapid bedside diagnosis [Ozomena et al., 2004; Schalk et al., 2004]. Ozomena et al. [2004] reported that the nested PCR method proved to be 10 to 100 times more sensitive than TaqMan PCR method, but they preferred TaqMan RT-PCR because of its advantages of contamination control, automation, and real-time quantitative features. The sensitivity of the system depends on the selection of primers and probe alignments [Ozomena et al., 2004].

LAMP was developed to amplify the target DNA without any temperature shifts for denaturation, annealing, and extension. LAMP has been applied for the detection of many kinds of infectious agents, mainly for the DNA virus of human herpesvirus (HHV) 6 and 7, varicella-zoster virus (VZV), or the bacterial genome [Iwamoto et al., 2003; Kuboki et al., 2003; Maruyama et al., 2003; Ihira et al., 2004; Okamoto et al., 2004; Yoshikawa et al., 2004]. Yoshikawa et al. [2004] reported that the VZV genome was amplified with a detection limit of 500 copies by LAMP. Recently, RT-LAMP method was reported for the detection of West Nile virus [Parida et al., 2004], SARS corona virus [Thai et al., 2003], and mumps virus [Okafuji et al., 2005]. SARS coronavirus was detected with high sensitivity of 0.01 pfu detection limit by RT-LAMP and 100-fold higher sensitivity of nested RT-PCR system. We developed RT-LAMP for the detection of the measles virus genome and compared the sensitivity of RT-LAMP with that of RT-PCR for the detection of measles virus. Measles RT-LAMP had 10-fold higher sensitivity than nested RT-PCR and the detection limit was approximately 0.01 TCID 50, 30–100 copies, similar to SARS RT-LAMP system. The measles virus genome was detected in 49 of 50 stored samples by RT-LAMP but in 44 by nested RT-PCR. It was also efficiently amplified from clinical samples of NPS, PMBC, and sera. Genotypes A, C1, D3, D5, and H1 were amplified without any differences in the detection limits. We could not examine all 22 genotypes. From the results of sequence alignments in Figure 5, several mutations were observed in eight primer regions and some were located at the 3’ end of each primer. It might influence the sensitivity of RT-LAMP, but we could deal with it by minor-modification of primers. RT-LAMP procedure is simple operation in a single tube and time-saving and we can obtain the results within 1 hr after the extraction of the virus genome. LAMP method for the detection of the genome of pathogenic agents is a useful tool in hospital-based rapid diagnosis. We calculated the quantity of genome in the sample by monitoring the spectrophotometric value and there was a linear correlation between the genome quantity and reaction time to reach the threshold. The quantitative RT-LAMP will contribute much to the better understanding about the different pathophysiology of virus infection [Okafuji et al., 2005].

The LAMP system has clinical benefits of high sensitivity, specificity, rapidness, and simplicity, which are required for its usage as a rapid diagnostic tool. This system will no doubt come into wide use as a diagnostic tool.

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REFERENCES

Afzal MA, Osterhaus ADME, Cosby SL, Jin L, Beeler J, Takeuchi K, Kawashima H. 2003. Comparative evaluation of measles virus-specific RT-PCR methods through an international collaborative study. J Med Virol 70:171–176.

Griffin DE, Bellini WJ. 2001. Measles virus. In: Fields virology, 4th edn. Philadelphia: Lippincott Williams & Wilkins. pp 1401–1441.

Helfand RF, Kim DK, Gary HE, Jr., Edwards GL, Bisson GP, Papania MJ, Heath JL, Schaff DL, Bellini WJ, Reedd SC, Anderson L. 1998. Nonclassic measles infections in an immune population exposed to measles during a college bus trip. J Med Virol 56:337–341.

Ihira M, Yoshikawa T, Enomoto Y, Akinoto S, Obashi M, Suga S, Nakamura N, Otake T, Nishiyama Y, Notomi T, Ohta Y, 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.

Iwamoto T, Sonobe T, 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.

Kobune F, Sakata H, Sugura A. 1990. Marmoset lymphoblastoid cells as a sensitive host for isolation of measles virus. J Virol 64:700–705.

Kuboki N, Inoue N, Sakurai T, Di Cello F, Grab DJ, Suzuki H, Sugimoto C, Igarashi I. 2003. Loop-mediated isothermal amplification for detection of African trypanosomes. J Clin Microbiol 41:5517–5524.

Kumada A, Komase K, Nakayama T. 2004. Recombinant measles AIK-C strain expressing current wild-type hemagglutinin protein. Vaccine 22:309–316.

Lievan FA, Papania MJ, Helfand RF. 2004. Lack of evidence of measles virus shedding in people with inapparent measles virus infections J Infect Dis 189:S165–S170.

Maruyama F, Kenzaka T, Yamaguchi N, Tani K, Nasu M. 2003. Detection of bacteria causing the stx2 gene in situ loop-mediated isothermal amplification. Appl Environ Microbiol 69:5023–5028.

Mori Y, Kitao M, Tomita N, Notomi T. 2004. Real-time turbidimetry of LAMP reaction for quantifying template DNA. J Biochem Biophys Methods 59:145–157.

Mossong J, Nokes DJ, Edmunds WJ, Cox MJ, Ratnam S, Muller CP. 1999. Modeling the impact of inapparent measles transmission in vaccinated populations with waning immunity. Am J Epidemiol 150:1238–1249.

Nagamine K, Hase T, Notomi T. 2002. Accelerated reaction by loop-mediated isothermal amplification using loop primers. Mol Cell Probes 16:223–229.

Nakayama T, Mori T, Yamaguchi S, Sonoda S, Asamura S, Yamashita R, Takeuchi Y, Urano T. 1995. Detection of measles virus genome directly from clinical samples by reverse transcriptase-polymerase chain reaction and genetic variability. Virus Res 35:1–16.

Nakayama T, Zhou J, Fujino M. 2003. Current status of measles in Japan. J Infect Chemother 9:1–7.

Notomi T, Okayama H, Masubuchi H, Yonekawa T, K. Watanabe K, Nakayama T, Mori Y, Kitao M, Tomita N, Notomi T. 2003. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res 28:e63.

Okafuji T, Yoshida N, Fujino M, Motegi Y, Ibara T, Ota Y, Notomi T, Nakayama T. 2005. Rapid diagnostic method for the detection of mumps virus genome by loop-mediated isothermal amplification. J Clin Microbiol 43:1625–1631.

Okafuji T, Yoshida N, Fujino M, Motegi Y, Ibara T, Ota Y, Notomi T, Nakayama T. 2005. Rapid diagnosis of human herpesvirus 6 infection by a novel DNA amplification method, loop-mediated isothermal amplification. J Clin Microbiol 42:140–145.
Ozoemena LC, Minor PD, Afzahl MA. 2004. Comparative evaluation of measles virus specific TaqMan PCR and conventional PCR using synthetic and natural RNA templates. J Med Virol 73:79–84.

Parida M, Posadas G, Inoue S, Hasebe F, Morita K. 2004. Real-time reverse transcription loop-mediated isothermal amplification for rapid detection of West Nile virus. J Clin Microbiol 42:257–263.

Schalk JAC, Van den Elzen C, Ovelgonne H, Baas C, Jongen PMJM. 2004. Estimation of the number of infectious measles viruses in live virus vaccine using real-time PCR. J Virol Methods 117:179–187.

Stein CE, Birmingham M, Kurian M, Duclos P, Strebel P. 2003. The global burden of measles in the year 2000—a model that uses country-specific indicators. J Infect Dis 187:S8–S14.

Takahashi M, Nakayama T, Kashiwagi Y, Takami T, Sonoda S, Yamanaka T, Ochiai H, Ihara T, Tajima T. 2000. A single genotype of measles virus is dominant whereas several genotypes of mumps virus are co-circulating. J Med Virol 62:278–285.

Thai HTC, Le MQ, Vuong CD, Parida M, Minekawa H, Notomi T, Hasebe F, Morita K. 2004. Development and evaluation of a novel loop-mediated isothermal amplification method for rapid detection of severe acute respiratory syndrome coronavirus. J Clin Microbiol 42:1956–1961.

WHO. 2001. Standardization of the nomenclature for describing the genetic characteristics of wild-type measles viruses. Wkly Epidemiol Rec 76:241–248.

WHO. 2002. WHO-UNICEF joint statement on strategies to reduce measles mortality worldwide. Wkly Epidemiol Rec 77:224–228.

Wild TF, Malvoisin E, Buckland R. 1991. Measles virus: Both haemagglutinin and the fusion glycoproteins are required for fusion. J Gen Virol 72:439–442.

Yamaguchi S. 1997. Identification of three lineages of wild measles virus by nucleotide sequence analysis of N, P, M, F and L genes in Japan. J Med Virol 52:113–120.

Yoshikawa T, Ihira M, Akimoto S, Usui C, Miyake F, Suga S, Enomoto Y, Suzuki Y, Nishiyama Y, Asano A. 2004. Detection of human herpesvirus 7 DNA by loop-mediated isothermal amplification. J Clin Microbiol 42:1348–1352.

Zhou J, Fujino M, Nakayama T. 2003. H1 genotype of measles virus was detected in outbreaks in Japan after 2000. J Med Virol 70:642–648.