DOI: 10.1111/j.1365-2362.2010.02427.x

ORIGINAL ARTICLE

M-specific reverse transcription loop-mediated isothermal amplification for detection of pandemic (H1N1) 2009 virus

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ABSTRACT

Background Since the initial outbreak in March 2009, the novel pandemic (H1N1) 2009 virus has affected individuals worldwide and caused over 18 138 deaths. There is an urgent need for the development of an easy, accurate and simple method for the diagnosis of this novel pandemic virus.

Design Reverse transcription loop-mediated isothermal amplification assay (RT-LAMP) with primers targeting the M segment was established for the rapid differential diagnosis of pandemic (H1N1) 2009 virus. The performance of this assay was characterized using 111 clinic nasopharyngeal swabs, and the diagnosis accuracy was compared with real-time reverse transcription PCR (RRT-PCR) and virus isolation, the latter being the reference standard.

Results This method successfully detected pandemic (H1N1) 2009 virus with a detection limit of approximately 20 copies of the target RNA per reaction, which is a comparably sensitivity to the RRT-PCR assay. Furthermore, this assay was able to discriminate pandemic (H1N1) 2009 virus from seasonal influenza viruses, such as H1N1 and H3N2, and other respiratory viruses (parainfluenza type 2 and 3, adenovirus, echovirus 7, and coxsackievirus A10). Based on validation by virus isolation, the specificity and sensitivity of this M-specific RT-LAMP assay were 100% and 98·25%, respectively. Moreover, the RT-LAMP amplification of most positive samples (46 out of 56) was achieved in < 20 min.

Conclusions This is an accurate and fast analysis system suitable for general diagnostic laboratories with only limited equipment, e.g. first-line health care centre. This assay will help clinicians and public health officials to react effectively during an outbreak.

Keywords Diagnosis accuracy, nasopharyngeal swabs, pandemic (H1N1) 2009 virus, real-time reverse transcription PCR, RT-LAMP.

Eur J Clin Invest 2010

Introduction

In March 2009, a novel swine-origin pandemic (H1N1) 2009 virus emerged in Mexico and spread rapidly via human-to-human transmission. On June 11 2009, the World Health Organization (WHO) raised the level of the influenza pandemic alert to Phase 6, indicating ongoing pandemic transmission. As of 30 May 2010, the pandemic (H1N1) 2009 virus has affected worldwide more than 214 countries and caused over 18 239 deaths [1]. Early detection of infected patients is an effective way to counteract the spread of this type of outbreak; it facilitates the clinical management of patients and also provides important information that is useful for epidemiological surveillance and risk assessment. These factors highlight the pressing need for the prompt development of adequate methods for the diagnosis of the novel influenza virus.

Real-time reverse transcription PCR (RRT-PCR) is the method of choice for clinical confirmatory diagnosis of pandemic (H1N1) 2009 virus [2]. The limit of detection achieved can be as low as 10 copies of RNA per reaction [3]. However,

because of its high complexity and the need for expensive equipment, not all laboratories have the infrastructure to perform the RRT-PCR test. Alternatively, rapid antigen tests have been widely used for the quick screening of influenza infection patients at first-line health care centres. Recently, the accuracy of commercially available rapid antigen detection tests was evaluated using real-time PCR as the gold standard. Drexler *et al.* [4] reported that the sensitivity of one commercial kit was about 11% for pandemic (H1N1) 2009 virus, despite this kit having higher sensitivities of 37·5% and 51% when detecting seasonal influenza viruses subtype H1N1 and H3N2, respectively. The limit of detection of various rapid antigen tests was also evaluated using two cultured viruses, i.e. A/California/4/09 (H1N1) and A/HK/415742/09 (H1N1), and the analytical sensitivity of the five test kits for pandemic (H1N1) 2009 virus was a tissue culture infectious dose 50 ($TCID_{50}$) $\log_{10}$ 3·3–4·7, which is equivalent to $\log_{10}$ 6·5–7·3 copies mL^{-1} of the M gene [5]. This should be compared with the PCR-derived method with a sensitivity ranging from 10 to 100 copies target RNA per reaction [3]. It is clear that the major drawback of the rapid antigen detection kits is poor sensitivity, which may produce misleading results in clinical practice.

Loop-mediated isothermal amplification (LAMP) is a novel method that amplifies DNA with high sensitivity and specificity [6]. Unlike other PCR-based techniques, this approach uses *Bst* DNA polymerase, which has high strand displacement activity, and therefore denaturation of the double-stranded template is not required for synthesis of new amplicons. Thus, the continuous amplification reaction can be carried out under isothermal conditions without a specialized thermocycler. This is especially useful in resource-limited situations. Since LAMP was first described in 2000, it has been successfully applied to the detection of many pathogens including a number of RNA viruses [7–14].

In the present study, considering the genetic stability of the area, the region of the matrix (M) gene unique to pandemic (H1N1) 2009 virus was chosen as the target for RT-LAMP amplification. The performance of the RT-LAMP approach was tested using 111 clinic nasopharyngeal swabs collected during 2009. In order to exclude cross-reactivity, the specificity of the pandemic (H1N1) 2009 virus LAMP test was evaluated using seasonal influenza virus subtypes H1N1 and H3N2, as well as other respiratory viruses. Finally, the specificity and sensitivity of the pandemic (H1N1) 2009 virus RT-LAMP analysis were compared with those of RRT-PCR.

Materials and methods

Reporting of the study conforms to STARD and the broader EQUATOR guidelines [15,16].

Viruses and virus isolation in cell cultures

The reference strain for pandemic (H1N1) 2009 influenza viruses (GenBank GU324338–GU324345) was isolated from the nasopharyngeal swab of a healthy pig and amplified in 10-day-old embryonated hen's eggs (specific pathogen-free, SPF) purchased from Department of Experimental Animal and Research of Animal Health Research Institute (AHRI), Taiwan. The titres of the influenza viruses were measured by haemagglutinin (HA) assay (expressed as HA unit) based on haemagglutination activity mediated by viral HA protein [17].

The identification of viruses from clinic swabs was definitively diagnosed based on virus isolation. Briefly, the nasopharyngeal swabs submitted to Virology Laboratory were kept in 2 mL of virus transport medium. In total, 200 μL of the transport medium was inoculated into five types of cultured cells (MRC-5, Hep-2, MDCK, MK-2, RD cells) that are susceptible to influenza virus infection and that of other respiratory viruses), and the cells were cultured for 14 days (or until cytopathic effects were observed). The identity of the viruses had been verified by immunofluorescence assay or RRT-PCR followed by automated sequencing.

Clinical samples

Altogether 111 RNA samples extracted from clinical nasopharyngeal swabs were used in this study, and these were initially diagnosed as suspected influenza virus infection cases by rapid antigen test and then were submitted to the China Medical University Hospital, Taichung, Taiwan, for definitive diagnosis. Among those, 57 were regarded as being pandemic (H1N1) 2009 positive by virus isolation using cell culture, and subsequently this identification of the resulting viruses was confirmed by RRT-PCR followed by automated sequencing.

Extraction of RNA from virus stocks or clinic samples

Except for the reference strain of pandemic (H1N1) 2009 virus, RNA from human influenza viruses and other respiratory viruses used in this study was directly extracted from clinic samples (the presence of target viruses being confirmed as described in the previous paragraph). Briefly, total RNA was extracted from the 0·2 mL of cultured virus stock, i.e., pandemic (H1N1) 2009 influenza (titre: 2^5 HA) or 0·14 mL of clinic specimen in viral transport medium using a QIAamp viral RNA mini kit (Qiagen, Hilden, Germany) according to the instructions of manufacturer.

In vitro transcription and quantification

To obtain a quantitative RNA standard to allow comparison of the sensitivity of the RT-LAMP and RRT-PCR assays, a plasmid bearing the intact coding region of the M gene was generated. In brief, the M gene was amplified from RNA of pandemic

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(H1N1) 2009 virus by a one-step RT-PCR kit (Bertec, Taipei, Taiwan) using the primer set M (sequences: F- AGCAAAAGC-AGGTAGATATT; R- AGTAGAAACAAGGTAGTTT). The 1027-bp amplicon was then cloned into the vector PCR 2.1-TOPO® (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The plasmid was confirmed by automated sequencing (Mission Biotech, Taipei, Taiwan). Plasmid (10 μg), linearized by the restriction enzyme *Hin*d III (BioLabs NEB, Ipswich, MA, USA), was then *in vitro* transcribed by T7 RNA polymerase (Promega, Madison, WI, USA) followed by treatment with DNase I (Invitrogen) to remove the DNA template. The purified RNA transcript was quantified by densitometer after gel electrophoresis by comparing the M gene with a standard sample at a known concentration. The target RNA copy number was then calculated by the following formula: copies μL^{-1} = concentration of plasmid in gram μL^{-1}/(RNA length 1027 nucleotides $\times$ 340) $\times$ 6·02 $\times 10^{23}$ (i.e. 1·7 $\times 10^{11}$ copies μL^{-1}).

RT-LAMP

Primers were designed to specifically target the matrix (M) gene. The nucleotide sequences of human isolates of pandemic (H1N1) 2009 virus were obtained from GenBank and were aligned with those of endemic strains and subtypes of influenza virus to identify potential target regions by GeneDoc software (Fig. 1). All the primers used in this study were synthesized by Mission Biotech. Primers for RT-LAMP are approximately located between 650 and 830 nucleotides in M gene; the detail positions of each primer are shown in Fig. 1. RT-LAMP was carried out using the Loopamp® RNA Amplification kit (Eiken Chemical Co., Ltd., Tokyo, Japan) including 2 μL RNA, 5 pmol each of the primers F3 (5′-CAGACTAGGCAGATGGTAC-3′) and B3 (5′-TCAGGTGCAAGATCCCAATG-3′), 20 pmol each of the primers LF (5′-AGACCAGCACTGGAGCTAGG-3′) and LB (5′-GATGCAGCGATTCAAGTGATC-3′), 40 pmol each of the primers FIP (5′-GCCTGCAAATTTTCAAGAAGGTCTTTTG-CAATGAGAACTATTGGGACTC-3′) and BIP (5′-CCAGAA-GCGAATGGGAGTGCTTTTATTTGCTGCAATGACGAGAG-3′) as described previously [18]. Importantly, the M-specific RT-LAMP was conducted blind to the virus isolation results, which were used as the gold standard for evaluating accuracy in this study. All the RT-LAMP reactions were conducted at 63 °C for 45 min and inactivated at 80 °C for 5 min. The RT-LAMP reactions were monitored using a LA-320C Loopamp® Real-time Turbiditymeter (Eiken Chemical Co., Ltd., Tokyo, Japan) [19] and analysed by gel electrophoresis (2% agarose dissolved in Tris–acetate–EDTA buffer; 40 mM Tris acetate and 1 mM EDTA).

Real-time RT-PCR

TaqMan® real-time RT-PCR was conducted following the recommendations of the CDC protocol for real-time RT-PCR of influenza A (H1N1) [2]. For confirmation of pandemic (H1N1) 2009 infection, the InfA, SW InfA, SW H1 and RnaseP primers per probe sets were used. For comparison of the sensitivity with RT-LAMP assay, the primers per probe set (InfA) targeting the M gene was used [2]. The thermocycler conditions followed the instructions in the CDC protocol. Briefly, for diagnosis purpose, 5 μL of the extracted RNA was used as template per reaction, and the RRT-PCR assay was performed using a LightCycler 480 Instrument (Roche Diagnostics, Mannheim, Germany). The results were analysed by LightCycler 480 Gene Scanning Software Version 1.0 software. A sample where the growth curve crossed the threshold line within 40 cycles ($Cp < 40$) was considered to be positive.

Nested RT-PCR

The nested RT-PCR was conducted using a one-step RT-PCR kit (Bertec) according to the manufacturer's instructions. Briefly, the outer primer set used for the one-step reverse transcription and the first run of nested PCR were as follows: NP-1085f (5′-GTMTCAAGYTTCATYAGAGG), NP-1565r (5′-AGTAGAAACAAGGGT ATT TTTC). The thermocycling conditions for amplification were as follows: 42 °C (40 min), 95 °C (2 min) followed by 35 cycles of denaturation (95 °C, 30 s), annealing (50 °C, 40 s) and extension (72 °C, 40 s); at the end of cycling there was a final extension (72 °C, 7 min). The resulting products from the first PCR were then used for nested PCR amplification with the inner primer set: AI-1200f (5′-CAGRTACTGGGCHATAAG RAC) and AI-1529r (5′-GCA-TTGTCTCCGAAGAAATAAG). The thermocycling conditions were the same as for the first run PCR, except that the annealing temperature was raised to 55 °C.

Statistical analysis

The agreement between the results of RRT-PCR assays and the results of the RT-LAMP assays was evaluated by calculating the Kappa statistic [20]. The Kappa (κ) value can be calculated as the agreement beyond chance divided by the amount of agreement possible beyond chance, i.e. (observed agreement % − expected agreement %)/(1 − expected agreement %). The κ values can be interpreted in the following qualitative manner: 0·0–0·2: slight agreement, 0·2–0·4: fair agreement, 0·4–0·6: moderate agreement, 0·6–0·8: substantial agreement, 0·8–0·1: almost perfect agreement.

Results

Differential detection of the pandemic (H1N1) 2009 virus M-specific RT-LAMP assay

Based on the genetic stability of the region, segment 7 encoding the matrix protein was chosen as the target for LAMP amplification. Based on the possibility of a cross-reaction with other

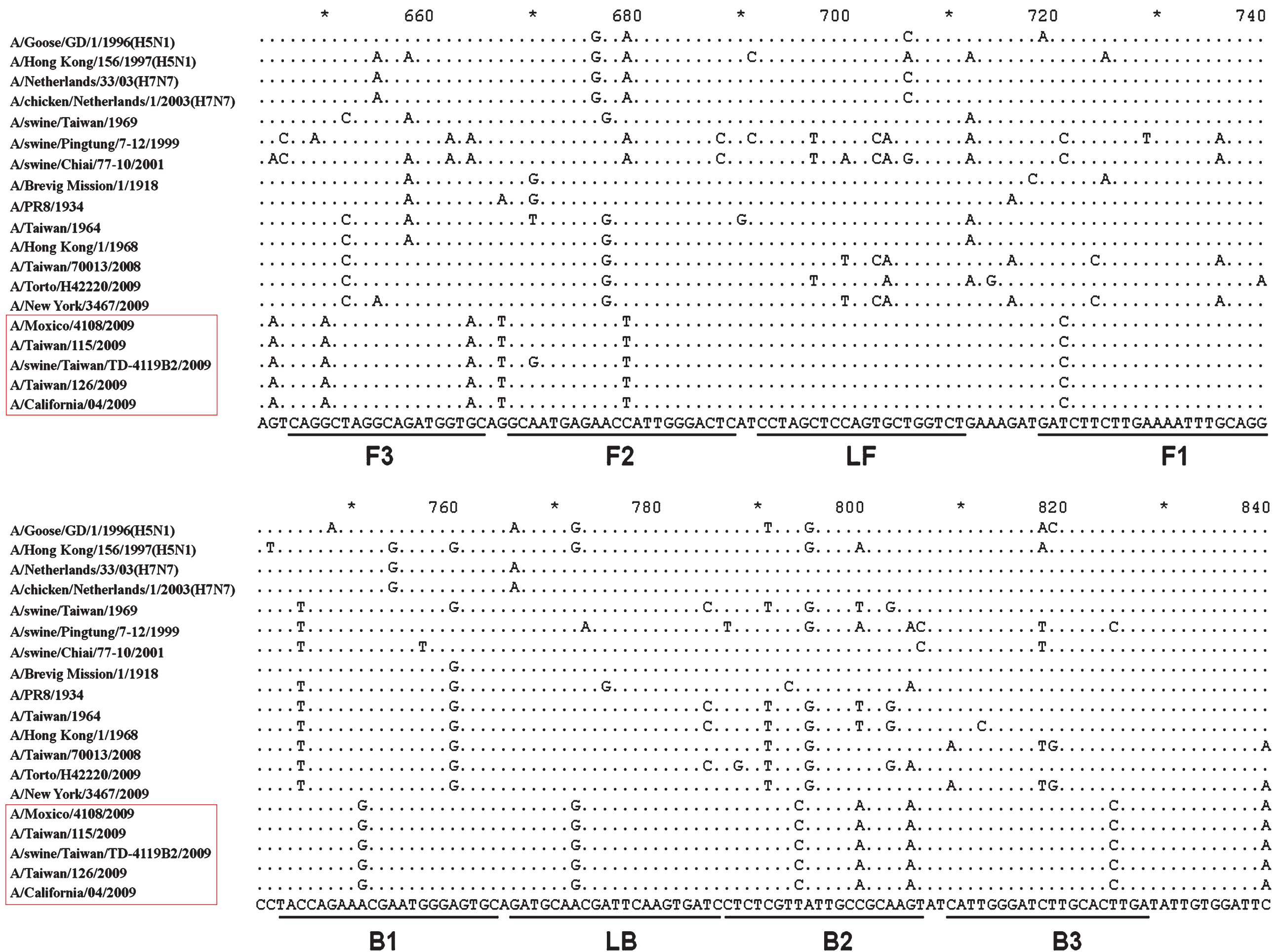

Figure 1 Sequence alignment of different influenza viruses and the location of the primers for the M-specific reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay. The M gene sequences of the pandemic (H1N1) 2009 virus strains marked in the square were aligned with those of pandemic Spanish influenza H1N1 (A/Brevig Mission/1/1918), seasonal type A influenza H1N1 (A/New York/3467/2009, A/Mexico/4108/2009, A/TW/70013/2008, A/PR8/1934), H2N2 (A/TW/1964) and H3N2 (A/Toronto/H42220/2009, A/HK/1/1968), three classic swine influenza strains (A/swine/Taiwan/1969, A/Pingtung/7-12/1999, A/Chiai/77-10/2001) and avian influenza subtypes H5N1 (A/Goose/GD/1/1996, A/Hong Kong/156/1997) and H7N7 (A/Netherlands/33/03, A/chicken/Netherlands/1/2003). Primers for the M-specific RT-LAMP assay were designed within the most conserved region for pandemic (H1N1) 2009 virus H1N1 and the most divergent region for the other viruses (nucleotides 640–850). The consensus sequence is shown at the bottom of each panel, dash lines represent sequence that is identical to the consensus sequence, and the locations of the primers are indicated by underlines.

influenza strains, the full-length M gene sequences of avian influenza (2002 isolates), human endemic influenza (678 isolates), swine influenza (179 isolates) and pandemic (H1N1) 2009 influenza viruses (143 isolates) were obtained from GenBank. After an initial phylogenetic analysis, a number of viruses from each cluster were selected for further sequence analysis. Sequence alignment of recent pandemic (H1N1) 2009 virus strains with human seasonal influenza viruses subtypes H1N1 and H3N2, pandemic Spanish influenza H1N1, avian subtypes (H5N1, H7N7) and swine influenza subtypes H1N2, H3N1 and H3N2 revealed that a region located from nucleotide 640 to nucleotide 850 is conserved among the pandemic (H1N1) 2009 virus strains, but is highly variable between the pandemic (H1N1) 2009 virus and other subtypes/different origin influenza viruses. Based on these results, a panel of primers for differential diagnosis of pandemic (H1N1) 2009 virus were designed to target this region (Fig. 1). The performance of this M-specific RT-LAMP assay was tested with RNA extracted from pandemic (H1N1) 2009 virus propagated in embryonic chicken eggs. In addition, the specificity of the assay was

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assessed retrospectively using seasonal influenza A, H1N1 and H3N2 samples, and a panel of commonly encountered respiratory viruses, namely parainfluenza type 2, parainfluenza type 3, adenovirus, coxsackievirus A10 and human echovirus 7. As shown in Fig. 2, a distinct laddering pattern of LAMP products was only obtained from the pandemic (H1N1) 2009 virus samples and not the other viral specimens, indicating the successful detection of pandemic (H1N1) 2009 virus H1N1 without cross-reactivity with seasonal influenza A and the other respiratory viruses. The sensitivity and time kinetics of the M-specific RT-LAMP amplification were initially characterized using serial dilutions of cultured pandemic (H1N1) 2009 virus. As illustrated in Fig. 3a, positive amplification was observed when 2^5 HA units of influenza virus were diluted 10^{-6} fold.

Sensitivity of M-specific RT-LAMP compared with RRT-PCR

The sensitivity of the M-specific RT-LAMP assay was then compared to real-time RT-PCR (RRT-PCR) using M-specific

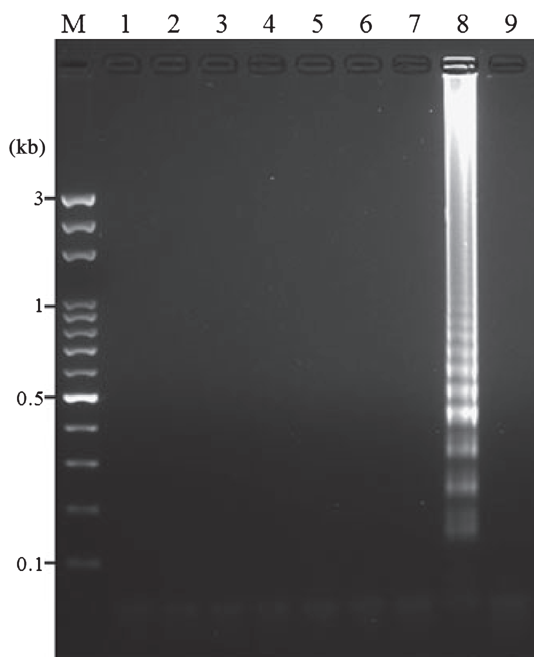

Figure 2 Evaluation of the specificity of the M-specific reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay. The specificity of the M-specific RT-LAMP assay was tested on pandemic (H1N1) 2009 virus H1N1 (lane 8), other human influenza A viruses, subtype H1N1 (lane 1) and H3N2 (lane 2), as well as other respiratory viruses, namely parainfluenza type 2, parainfluenza type 3, adenovirus, coxsackievirus A10, human echovirus 7 (lanes 3–7), and negative control (lane 9). M: DNA marker.

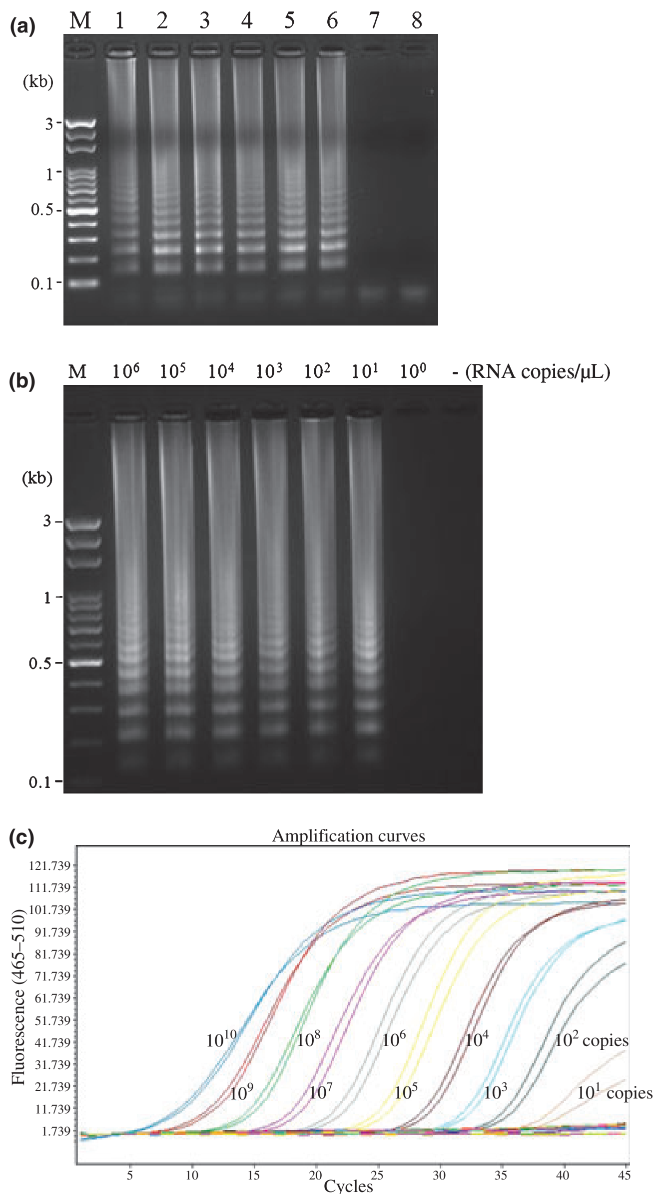

Figure 3 Sensitivity of the M-specific reverse transcription loop-mediated isothermal amplification (RT-LAMP) and real-time RT-PCR assays. Sensitivity of LAMP was initially tested on viral RNA extracted from 10-fold serial dilutions of cultured pandemic (H1N1) 2009 virus consisting of 2^5 HA units viruses over the range 10^{-1}–10^{-7} (samples 1–7). Positive amplification was observed in samples 1–6, but not in sample 7 (10^{-7} diluted viruses) and sample 8 (which included all reagents but without RNA template as the negative control) (a). The sensitivity of M-specific RT-LAMP was further analysed with *in vitro* transcribed RNA containing the full length of the M gene. M transcript was diluted to 10^{10}–10^{0} copies μL^{-1} and was used as standard template to evaluate the sensitivity of RT-LAMP (b) and RRT-PCR (c) assays.

primers/probe following the CDC protocol. The *in vitro* transcribed segment 7 RNA was quantified and then 10-fold serially diluted to give final concentrations between 10^0 and 10^8 copies μL^{-1}. The end point detection limits for the RT-LAMP assay was approximately 20 copies per reaction, which is a comparable sensitivity to that of the RRT-PCR assay (10 copies per reaction) (Fig. 3b,c).

Evaluation of the M-specific RT-LAMP assay using 111 clinical nasopharyngeal swabs

To assess the performance of M-specific RT-LAMP, we retrospectively screened 111 RNA samples extracted from influenza virus-suspected clinical specimens submitted to the China Medical University Hospital during the summer of 2009. All the samples were initially tested using pandemic (H1N1) 2009 virus-specific RRT-PCR in the Department of Medical Technology and then were definitively confirmed by virus isolation conducted by the staff of the Clinic Virology Laboratory of China Medical University Hospital. The virus isolation result served as the gold standard for evaluating accuracy in this study. Using the RT-LAMP analysis, 56 out of 57 pandemic (H1N1) 2009 virus samples, as confirmed by virus isolation, tested positive, which gives a sensitivity of 98·25%. In terms of specificity, none of the 54 pandemic (H1N1) 2009 virus-negative samples were found to be positive by RT-LAMP analysis, which is a specificity of 100% (Table 1). Importantly, all the positive amplifications by RT-LAMP were achieved in < 35 min, and 46 of them (82·1%) were amplified as positive within 20 min (Fig. 4). When the M-specific RT-LAMP assay was compared with the RRT-PCR assay, among the 57 pandemic (H1N1) 2009 virus-positive samples by virus isolation, five of them were detected negative by RRT-PCR (Table 1). The disagreement between these analysis methods was then validated by nested PCR, and the results indicated the five samples indeed were infected with pandemic (H1N1) 2009 virus (data not shown). In addition, the one pandemic (H1N1) 2009 virus-negative sample, as confirmed by both viral isolation and RT-LAMP, was detected as positive by RRT-PCR. This particular sample was further identified as subtype H3N2 by virus isolation. Notwithstanding the above, the high consistency of M-specific RT-LAMP method in terms of both viral isolation and RRT-PCR gave κ values of 0·9820 and 0·8919, respectively.

Table 1 A comparison of the assay results by M-specific RT-LAMP assay, RRT-PCR assay and virus isolation using 111 clinical specimens

	M-specific LAMP		RRT-PCR	
	Positive	Negative	Positive	Negative
Pandemic (H1N1) 2009*				
Positive (*n* = 57)	56	1	52	5
Negative (*n* = 54)	0	54	1	53
Sensitivity (%)	98·25		91·22	
Specificity (%)	100		98·10	

RT-LAMP, reverse transcription loop-mediated isothermal amplification.
*Defined by viral isolation followed by validation using RRT-PCR.

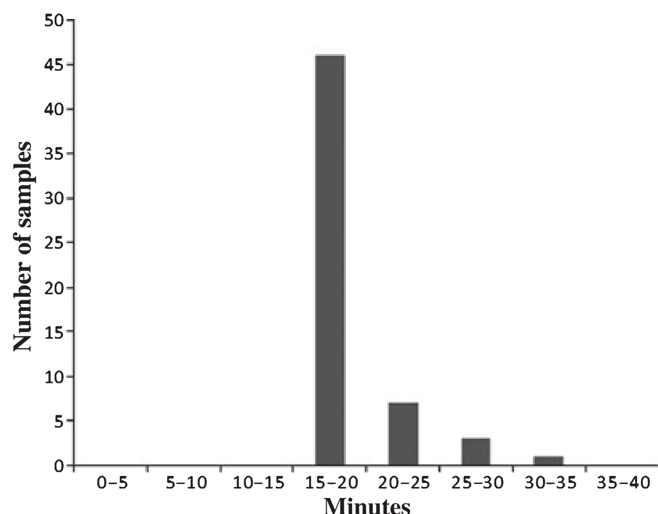

Figure 4 The reaction time of the M-specific reverse transcription loop-mediated isothermal amplification assay as performed on 56 pandemic (H1N1) 2009 virus clinical samples. The amplification of the pandemic (H1N1) 2009 virus samples was real-time monitored by turbiditymeter. The reaction of 46 of 56 samples had reached a maximum by 20 min after initiation of the reaction.

Discussion

Real-time reverse transcription PCR (RRT-PCR) is the method that has been routinely used for the detection of pandemic (H1N1) 2009 virus in reference laboratories and is considered to be the gold standard when evaluating the performance of other comparable methods. However, due to the assay's technical complexity and its high cost in equipment and reagents, RRT-PCR is not widely established in first-line care laboratories with limited equipment. A number of nucleic acid amplification assays have been described for endpoint detection of viruses. Among those, LAMP is well known for possessing superior isothermal reaction characteristics and is widely regarded as a simple, rapid, specific and cost-effective nucleic acid analysis method [7,10,12–14].

Very recently, Kubo *et al.* [21] established a HA-specific LAMP assay for the detection of pandemic (H1N1) 2009 virus with a sensitivity of 97·8% and a specificity of 100%. In this context, a reliable nucleic acid-based diagnosis method has to

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consider the genetic stability of the target RNA genome. Results from a comparison study of sequences deposited into public databases suggest that since April 2009 a range of genetic variation has arisen in H1N1 viruses [22]. As the envelope glycoproteins HA and neuraminidase (NA) are the major immunity-eliciting antigens, these are therefore the main targets for host-driven antigenic drift [23,24] and a higher frequency of variation in these genes can be expected. Supportive evidence from mutation trend analysis has revealed that both HA and NA are prone to significant change [25]. To circumvent the possibility of false negative results due to mutations in these genes, the RT-LAMP method in the present study was designed to detect the gene encoding the matrix protein, a type-specific antigen of influenza virus that is highly conserved and undergoes less evolutionary change [26,27]. Nevertheless, one false negative result was obtained out of 57 pandemic (H1N1) 2009 virus-positive samples identified by virus isolation when the clinical samples were tested with M-specific RT-LAMP. This finding occurred in two out of three experiment replicates with one out of three being positive. There are two possible explanations for the failure of the amplification. First, the RNA copy number of template might be limiting due to a low amount of pandemic (H1N1) 2009 virus in the specimen. Secondly, there might have been a poor yield of RNA when the specimen was extracted. This sample also tested negative by RRT-PCR, and therefore it is likely that amount of viral RNA present was the cause of the false negative interpretation. Despite this, the overall performance of the RT-LAMP analysis established in current study is highly consistent with both the virus isolation and RRT-PCR results.

It is worthy of note that, as the M segment of pandemic (H1N1) 2009 virus is phylogenetically close to the avian-like swine lineage, one might be concerned about cross-reactivity when using the M-specific RT-LAMP with other avian-origin influenza viruses. However, unlike swine influenza, avian influenza A viruses do not commonly circulate in humans, and therefore the chance of this potential misinterpretation is low. On the other hand, learning from our experiences during H5N1 outbreaks, any avian origin influenza virus detected in a human specimen indicates a cross-species infection, which means that special precautions need to be taken. Hence, any possible lack of specificity related to the M-based nucleic acid assay should not pose a particular problem for its routine use, and any human sample with a risk of being infected by avian influenza should also be tested using a H5N1-specific assay to obtain a definite diagnosis[28].

Currently, several commercially available rapid antigen detection tests are widely used for the first-line screening of influenza A infections in general practice or at central laboratories; however, an overall low sensitivity remains the major drawback of these tests [4,5,29]. Furthermore, a previous report has suggested that the antigen-based immunoassay system is unable to differentiate between pandemic (H1N1) 2009 virus and seasonal influenza A or even between subtypes H1 and H3 [5]. Thus, a definite diagnosis of pandemic (H1N1) 2009 virus still relies on RRT-PCR, which requires complex equipment that might not be available in a front line situation. The one-step M-specific RT-LAMP method described in this study is pandemic (H1N1) 2009 virus specific (100% specificity as validated by virus isolation), highly sensitive (as low as 20 copies), time effective (within 30 min post RNA extraction), cost-effective (< 5 US dollars per reaction) and requires only basic equipment (heating block and centrifuge). Taking into consideration the simplicity, rapidity, and accuracy of the M-specific RT LAMP assay, it is clear that it has potential to be established as a high throughput analysis system when substantial demands are placed on laboratories during an emerging pandemic outbreak. Furthermore, it can also be used in front line situations where immediate diagnosis is needed, but limited equipment is available.

Acknowledgements

This work was supported by grants from National Science Council (NSC 98-2313-B-005-015-MY3), China Medical University Hospital (DMR-99-126), and National Chung-Hsing University (CC99118A2). The authors thank Professor Ralph Kirby, in National Yang-Ming University, Taiwan for editorial assistance.

Author contributions

M-S Lee who specializes in nuclei acid amplification tests conducted RT-LAMP. H-C Shih and P-Y Chen participated in clinical sample collection. M-C Su, the senior clinic virologist, conducted the virus isolation under supervision of J-J Liu. M-C Deng and C-C Wu conducted the RRT-PCR. F-Y Lin, K-H Lin participated in the PCR confirmation.

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Received 30 July 2010; accepted 18 October 2010

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