Abstract
Most of the diseases caused by viral infection are found to be fatal, and the diagnosis is difficult due to confusion with other causative agents. So, a highly efficient molecular-based advance detection technique, i.e., loop-mediated isothermal amplification (LAMP) method, is developed for diagnosis of viral infections by various workers. It is based on amplification of DNA at very low level under isothermal conditions, using a set of four specifically designed primers and a DNA polymerase with strand displacement activity. This technique is found to be superior than most of the molecular techniques like PCR, RT-PCR, and real-time PCR due to its high specificity, sensitivity, and rapidity. Major advantage of LAMP method is its cost-effectiveness as it can be done simply by using water bath or dry bath. Here, in this review information regarding almost all the effective LAMP techniques which is developed so far for diagnosis of numerous viral pathogens is presented.

21.1 Introduction
Viruses exist in living system since living cells first evolved, so found wherever there is life exist. Viruses do not form fossils so the mode of their investigation/detection is only by using molecular techniques (Iyer et al. 2006). Most of the viruses are harmful to animals (http://en.wikipedia.org/wiki/Introduction_to_viruses) and cause a number of infectious diseases. Common human diseases caused by viruses include the common cold, influenza, chickenpox, and cold sores. Ebola, AIDS, avian influenza, and SARS are various serious diseases caused by viruses (Emiliani 1993). The relative ability of viruses to cause disease is known as virulence of virus. Viral diseases sometimes cause confusion with any other causative agent such as the possible connection between human herpesvirus six (HHV6) and neurological diseases such as multiple sclerosis and chronic fatigue syndrome (Thomas 2008). There is controversy over whether the bornavirus, previously thought to cause neurological diseases in horses, could be responsible
for psychiatric illnesses in human (Mandal 2013). According to Shors (2008) viruses transmit by two modes, i.e., vertical (from mother to child) and horizontal (from person to person). Vertical transmission include hepatitis B virus and HIV where the baby is born already infected with the virus. Varicella-zoster virus is another but rarest example of vertical transmission. Although this virus causes relatively mild infections in humans, it can be fatal to the fetus and newly born baby (Mandal 2013). Generally living populations are affected by horizontal transmission of viral infection where viral transmission occurs via exchange of blood and by sexual activity (HIV, hepatitis B, and hepatitis C), through mouth by exchange of saliva (Epstein-Barr virus), through contaminated food or water (norovirus), by breathing viruses in the form of aerosols (influenza virus) and by insect vectors such as mosquitoes (dengue) (Collier et al. 1998). Currently, 21 families of viruses are known to cause disease in humans (http://en.wikipedia.org/wiki/virus_classification). So due to broad spectrum of harmful infections/diseases caused by viruses, a rapid and accurate diagnosis is essential for timely therapeutic interventions (Barenfanger et al. 2000).

Several methods such as viral antigen, genomic sequences, and/or antibodies are used for the detection of viral infection. Viral isolation and serological assays are standard methods of viral diagnosis. Both viral isolation and serological testing require substantial time to obtain accurate final results. On the other hand, use of histological and biochemical methods for virus detection may sometimes fail to detect the virus antigen during the early stages of infection. Among various conventional methods, viral culture and antigenic detection (immunological detection) methods of viral detection are often costly, time-consuming, and less sensitive. Parida (2008) suggested that nucleic acid amplification is one of the most valuable tools in virtually all life science fields, including application-oriented fields such as clinical medicine in which diagnosis of infectious diseases, genetic disorders, and genetic traits is based on this novel method. Several amplification methods, namely, nucleic acid sequence-based amplification (NASBA), self-sustained sequence replication (3SR), strand displacement amplification (SDA), as well as polymerase chain reaction (PCR), have been invented in past days (Chan and Fox 1999). Nonquantitative PCR and immunological detection methods are usually only qualitative tests with limited sensitivity and cannot distinguish infective viruses (Enteric Viruses: Rapid Detection and Identification Methods. http://www.rapidmicrobiology.com/test-methods/Enteric-viruses.php). PCR is one of the conventional methods which are affordable, but it has some intrinsic limitations such as the requirement of rapid thermal cycling, insufficient specificity, and low-amplification efficiency (Mori et al. 2001). Nested PCR and multiplex PCR have been developed to address the need for rapid identification of viruses to serotype level with more accuracy (Ratcliff et al. 2007). Another novel technique, i.e., real-time PCR, is developed which in turn brought true quantization of target nucleic acids out of the pure research laboratory and into the diagnostic laboratory (Espy et al. 2006). Mackay et al. (2002) reported quantitative PCR (QPCR) assays as most established assay for the detection of viral load and therapy monitoring. Nucleic acid-based assays or real-time quantitative assay might eventually replace virus isolation and conventional RT-PCR as the new gold standard for the rapid diagnosis of virus infection in the acute-phase samples (Ratcliff et al. 2007). Besides, all these nucleic acid amplification methods have several intrinsic disadvantages of requiring either a high-precision instrument for amplification or a complicated method for detection of amplified products. The high cost of instruments required for performing the real-time assays restricted its use to laboratories with good financial resources (Parida et al. 2008).

To overcome all above limitations in viral diagnosis, a loop-mediated isothermal amplification (LAMP) method was developed as an alternative method for viral detection. LAMP is a powerful and novel nucleic acid amplification method that amplifies a few copies of target DNA with high specificity, efficiency, and rapidity under isothermal conditions, using a
set of four specially designed primers and a DNA polymerase with strand displacement activity (Notomi et al. 2000; Parida et al. 2008; Tomita et al. 2008). When detecting the RNA genome of a pathogen such as an RNA virus, LAMP has been merged with reverse transcription (RT) into RT-LAMP to allow nucleic acid amplification (Soliman et al. 2009). This technique requires only simple and cost-effective equipment amenable to use in clinical laboratories (Enomoto et al. 2005). Compared to PCR and real-time PCR, the LAMP has the advantages of reaction simplicity and detection sensitivity. It has been claimed that the LAMP method can detect as few as 100 copies of DNA template in blood samples, equal roughly to 5 parasites/μl (Han et al. 2007). This sensitivity is notably higher than any known immunochromatography-based RDTs, which are recommended by WHO as part of the global malaria control strategy (Long 2009). LAMP has potential applications for clinical diagnosis as well as surveillance of infectious diseases in developing countries without requiring sophisticated equipment or skilled personnel (Mori and Notomi 2009; Parida et al. 2008).

21.2 Background of LAMP Technique

Although the inception of LAMP refers back to 1998, the popularity of LAMP starts only after 2003 following emergence of West Nile and SARS viruses. This technique was first described and initially evaluated for detection of hepatitis B virus DNA by Notomi et al. (2000). First of all, LAMP has been applied to many kinds of pathogens causing food-borne diseases (Lukinmaa et al. 2004). LAMP kits for detecting Salmonella, Legionella, Listeria, verotoxin-producing Escherichia coli, and Campylobacter have been commercialized. Due to its advantages, later on, this method is successfully employed for detection of variety of bacteria, viruses, parasites, and fungus.

21.3 Advantages of LAMP Method over Conventional Methods

1. Amplifies a few copies of target DNA.
2. Easy to handle.
3. Higher sensitivity and specificity is attributed to continuous amplification under isothermal condition employing four primers recognizing six distinct regions of the target (Parida 2008).
4. High efficiency.
5. Rapid detection.
6. Cost-effective as it can be carried out with simplest equipment, i.e., dry bath/water bath (Enomoto et al. 2005).
7. Vast application area in detection of different types of microorganisms (detection of variety of bacteria, viruses, parasites, and fungus) (Endo et al. 2004; Okafuji et al. 2005).
8. Can detect RNA genome of a pathogen by reverse transcription using reverse transcriptase enzyme (Soliman et al. 2009).
9. LAMP product can be easily detected visually either by using SYBR Green I or by using turbidometer.
10. Free online software Primer-Explorer IV software program (http://primerexplorer.jp/e/) is available for LAMP primer design.

21.4 Detection of Viruses by LAMP Method

LAMP assay is also helpful in detection of RNA template by combo use of reverse transcriptase along with DNA polymerase (Notomi et al. 2000; Whiting and Champoux 1998). Due to this, LAMP has already found wide application in RNA virus detection, such as foot-and-mouth disease (Dukes et al. 2006), swine vesicular disease (Blomstrom et al. 2008), Taura syndrome (Kiatpathomchai et al. 2007), severe acute respiratory syndrome coronavirus (Hong et al. 2004),
orovirus (Fukuda et al. 2006), human papilloma virus (Hagiwara et al. 2007), cytomegalovirus (Reddy et al. 2010), human immunodeficiency virus (Hosaka et al. 2009), and H5N1 avian influenza virus (Jayawardena et al. 2007).

### 21.4.1 Human Viruses

LAMP method has been developed for detection of human viruses including chickenpox virus (Okamoto et al. 2004); mumps virus (Yoshida et al. 2008); Colorado tick fever virus (Lee et al. 2010); respiratory syncytial virus (Okamoto et al. 2004); enterovirus 71 (Shi et al. 2011); human immunodeficiency virus (HIV) causing acquired immune deficiency syndrome (AIDS) (Hosaka et al. 2009); pandemic (H1N1) 2009 virus (Lee et al. 2010); cytomegalovirus causing cytomegalovirus inclusion disease (Reddy et al. 2010); chikungunya virus (Parida et al. 2007); Japanese encephalitis virus (Parida et al. 2006); herpes simplex virus type 2 causing genital herpes (Enomoto et al. 2005); adult T-cell leukemia and hairy cell leukemia viruses; human T-cell lymphotropic-1 and human T-cell lymphotropic-2 retroviruses (Komiyama et al. 2009); foot-and-mouth disease virus (Chen et al. 2011); Epstein-Barr virus (EBV) (Iwata et al. 2006), influenza A and B viruses causing influenza (Ito et al. 2006); Ebola virus (Kurosaki et al. 2007); human papilloma viruses type 6, 11, 16 and 18 (Hagiwara et al. 2007); and HIV-1 virus (Curtis et al. 2009).

Mumps virus was circulating throughout the world, but in the United States the acceptance of MMR combined vaccine against measles, mumps, and rubella has reduced the number of patients suffering from mumps (Centers for Disease Control and Prevention 1998; Peltola et al. 1994). Mumps patients having high vaccine coverage are prone to suffering from secondary vaccine failure (SVF) (Pugh et al. 2002). Due to this critical problem, rapid detection of mumps virus by a fast diagnostic technique was necessary. Therefore, Okafuji et al. (2005) developed a fast LAMP assay for detection of mumps virus genome with a detection of 0.1 PFU along with the same sensitivity as RT-nested PCR within 60 min only. Similarly, Yoshida et al. (2008) developed RT-LAMP assay for mumps virus with a detection limit of 0.12 PFU/μl. According to a WHO report, approximately two billion people are infected with hepatitis B virus (HBV) and more than 350 million have a chronic HBV infection. Molecular diagnostic assays have provided insight into the pathogenesis and natural history of HBV infection and have facilitated detection of drug resistance (Lok et al. 2001; Corden et al. 2003). Hepatitis B virus (HBV) is responsible for hepatocellular carcinoma (a liver disease) in approximately 350–400 million people all over the world (Dienstag 2008). Cai et al. (2008) detected hepatitis B virus by Rf-LAMP and determined the lower detection limit of LAMP by Probit analysis at the 95 % detection level (210 copies/ml). So, Rf-LAMP was more precise in detecting low titers of the virus which makes it more useful than real-time PCR in clinical applications. Moslemi et al. (2009) also detected hepatitis B virus by LAMP method and observed that PCR sensitivity was up to 40 particles, while the LAMP sensitivity test was verified up to four particles.

Human respiratory syncytial virus (RSV) is a major causative agent of lower respiratory tract infections in children and adults worldwide. Children born preterm or with underlying cardiopulmonary disorders are at specially high risk of developing severe and lethal RSV respiratory tract infections (American Academy of Pediatrics 2003; Girard et al. 2005; Satjie et al. 1993, 1994). A reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay was developed by Shirato et al. (2007) to amplify the genome of RSV subgroups A and B, in order to improve current diagnostic methods for RSV infection. For RSV detection, primers were designed according to the highly conserved nucleotide sequence located in the matrix protein region of the nucleoprotein region. RT-LAMP can detect viral RNA in 10⁻¹ to 10⁻² PFU of RSV.

Generally, smallpox and AIDS viruses attack only humans, but in 1995 the first report of a chimpanzee being infected with HIV was reported. Reverse transcription loop-mediated
isothermal amplification assay was developed for rapid detection of HIV type 1 (group M) (Hosaka et al. 2009). The 100% detection limit of HIV-1 RT-LAMP was determined using a standard strain (WHO HIV-1 [97/656]) in octuplicate and found to be 120 copies/ml.

Hand-foot-and-mouth disease (HFMD) is a common infectious disease in young children and infants and is characterized by fever, ulcers in the mouth, and vesicles on the hands and feet. Two enteroviruses, i.e., human enterovirus 71 (EV71) and coxsackievirus A16 (CVA16), are responsible for this disease. Schmidt et al. (1974) reported that EV71 was first isolated from an infant with encephalitis in California in 1969. HFMD epidemics cause serious public health threats among infants throughout the world (Lee et al. 2009; McMinn 2002). Jiang et al. (2011) developed an RT-LAMP assay targeting the VP3 gene of EV71 and further evaluated with 40 clinical stool samples collected during the epidemics of HFMD in Guangxi province of China in 2009. This assay was found to be more sensitive and accurate, so it may become a useful alternative in resource-limited hospital laboratories and rural clinics for diagnosis of EV71. The sensitivity of the EV71-specific RT-LAMP is 0.01 PFU per reaction mixture, which is similar to the rRT-PCR assay. Based on 40 clinical samples from suspected HFMD cases, 92.9% diagnostic sensitivity was achieved with the RT-LAMP assay.

Out of 15 hemagglutinin subtypes and 9 neuraminidase subtypes, only 3 hemagglutinin (H1 to H3) and 2 NA (N1 and N2) subtypes have established stable lineages in the human population since the last century (Webby and Webster 2003). Highly contagious nature of influenza A viruses (Fraser et al. 2004) leads the disease rapidly spread in close communities. Poon et al. (2005) developed LAMP assay for detection of influenza A virus with detection limit of ten copies per reaction.

Novel H1N1 (letters refer to haemagglutinin and neuraminidase proteins on the surface of the virus; the numbers refer to slight variations in the forms of each protein) 2009 pandemic virus affected individuals worldwide and caused over 18,138 deaths since its initial outbreak in March 2009. Comparative study of sequences available into public databases suggests that since April 2009 a range of genetic variation has arisen in H1N1 viruses (Zhang et al. 2009). To eradicate the probability of false-negative results due to mutations in these genes, the RT-LAMP method was designed by Hiromoto et al. (2000) and Welman and Arora (2000) in order to detect the gene encoding the matrix protein, a type-specific antigen of influenza virus (highly conserved and undergoes less evolutionary change). Out of 57 pandemic (H1N1) 2009 virus-positive samples identified by virus isolation only one false-negative result was obtained when the clinical samples were tested with M-specific RT-LAMP. There are two possible explanations for the failure of the amplification. It was worthy to 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 chances of this potential misinterpretation are low. On the other hand, learning from previous 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 an H5N1-specific assay to obtain a definite diagnosis (Carr et al. 2009). The sensitivities of the pandemic (H1N1) 2009 virus HA-specific RT-LAMP assay and a TaqMan real-time reverse transcription PCR assay performed with the SW H1 primer/probe set (WHO 2009b) were compared by testing ten-fold serial dilutions of in vitro-transcribed target RNA (from 100 to 104 copies) in quadruplicate. Both assays were equally sensitive, and the detection limit was ten copies of target RNA per reaction volume. All the positive amplifications by the pandemic (H1N1) 2009 virus HA-specific RT-LAMP assay were
achieved in less than 40 min. The pandemic (H1N1) 2009 virus HA-specific RT-LAMP assay targets only the HA gene, so this RT-LAMP method has difficulty with the detection of pandemic (H1N1) 2009 viruses which have HA genes that are difficult to amplify even by rRT-PCR. This may be a limitation of the pandemic (H1N1) 2009 virus HA-specific RT-LAMP method. This limitation could be overcome by combining the pandemic (H1N1) 2009 virus HA-specific RT-LAMP assay with conventional RT-PCR for the matrix gene of influenza A virus (WHO 2009a) or by designing influenza A virus consensus RT-LAMP assay primers and performing the two RT-LAMP assays together. Based on above facts and data, there is an urgent need for the development of an easy, accurate, and simple method for the diagnosis of this novel pandemic virus (Lee et al. 2010). Lee et al. (2010) developed M-specific reverse transcription loop-mediated isothermal amplification for detection of pandemic (H1N1) 2009 virus. Based on validation by virus isolation, the specificity and sensitivity of this M-specific RT-LAMP assay were 100 % and 98.25 %, respectively. A newly emerged pandemic human influenza virus 2009 which is a triple reassortant including segments from swine, avian, and human influenza viruses (Garten et al. 2009; Shinde et al. 2009; Smith et al. 2009) has spread throughout the world. Kubo et al. (2010) developed an HA-specific LAMP assay for the detection of pandemic (H1N1) 2009 virus with 100 % specificity and 97.8 % sensitivity. This method was also considered as a novel molecular method for diagnosis of pandemic influenza in resource-limited settings.

In the early stages of ocular manifestations, clinical differentiation between patients of retinitis associated with CMV and other herpesvirus infections is often difficult (Knox et al. 1998). Viral retinitis is commonly caused by herpes simplex virus type 1 (HSV-1), HSV-2, varicella-zoster virus (VZV), cytomegalovirus (CMV), and occasionally by Epstein-Barr virus (EBV) (Madhavan et al. 2004). It is very important to differentiate CMV retinitis from HSV and VZV retinitis early in the course of infection because the therapeutic agent to be used for treatment differs from virus to virus (Madhavan et al. 2004). So LAMP assay was developed for their differentiation. Reddy et al. (2010) developed a simple and cost-effective LAMP assay for the rapid detection of CMV DNA in patients infected with viral retinitis. It is highly sensitive as its lower detection limit is ten copies of CMV DNA per microliter. Also it is a time-saving technique; the time required for amplification and detection of the product is about 75 min by the LAMP assay, whereas that for PCR is 4 h.

In recent years, emergence or reemergence of severe arboviral hemorrhagic fevers caused by mosquito-borne viruses such as dengue virus and chikungunya (CHIK) virus has been frequently reported in the Indian subcontinent. Parida et al. (2007) developed a rapid and real-time detection of chikungunya virus by RT-LAMP assay. RT-LAMP assay correctly identified 21 additional positive borderline cases from acute-phase patient serum samples that were missed by conventional RT-PCR (P < 0.0001) with a detection limit of 20 copies. Humans infected with CHIK virus produce an illness often characterized by sudden onset of fever, headache, fatigue, nausea, vomiting, rash, myalgia, severe arthralgia, and polyarthralgia (typical clinical sign of the disease is very painful). Self-limiting symptoms last from 1 to 10 days.

The most common cause of childhood viral encephalitis is Japanese encephalitis virus (JEV) in the world, causing an estimated 50,000 infections and 10,000 deaths annually. Several serological tests, such as the hemagglutination inhibition test, enzyme-linked immunosorbent assay (ELISA), serum neutralization techniques, and dot enzyme immunosassay, had been used for the detection of antibody for JEV infection (Burke et al. 1982; Soloman et al. 1998). For many years, the hemagglutination inhibition test had been employed, but this has various practical limitations. Besides being cumbersome, it requires paired serum samples and therefore cannot give an early diagnosis. In 2006, Toriniwa and Komiya standardized RT-LAMP for the rapid detection and quantification of JEV by targeting the E gene, but still the application of this novel gene amplification system for the clinical diagnosis of JE
patients during epidemic situations needs to be established through evaluation with a large number of clinical samples (Toriniwa and Komiya 2006). Further, Parida et al. (2006) also developed LAMP assay for detection of JEV, but this time particular importance is given to the substantial reduction in time required for the confirmation of results by RT-LAMP assay (in 30 min compared to 3–4 h in the case of RT-PCR). The RT-LAMP assay demonstrated exceptionally higher sensitivity compared to that of RT-PCR, with a detection limit of 0.1 PFU.

Dengue virus is a mosquito-borne flavivirus and the most widely prevalent arbovirus in tropical and subtropical regions of Asia, Africa, and Central and South America (Gubler 1997). Four distinct serotypes, i.e., DEN-1, DEN-2, DEN-3, and DEN-4, produce a spectrum of illness ranging from in apparent infection to moderate febrile illness and severe, fatal hemorrhagic disease. The three basic methods routinely practiced by most laboratories are virus isolation, characterization, detection of dengue virus-specific antibodies, and detection of genomic sequences by nucleic acid amplification techniques (Guzman and Kouri 1996; Innis et al. 1989; WHO 1997). Both virus isolation and PRNT assays are time-consuming and tedious and require more than a week for completion. Several investigators have reported on fully automatic real-time PCR assays for the detection of dengue virus in acute-phase serum samples (Callahan et al. 2001; Houng et al. 2001; Laue et al. 1999; Seah et al. 1995). During the past decade, various nucleic acid amplification techniques such as RT-PCR, nested PCR, Taqman real-time RT-PCR, SYBR Green I real-time RT-PCR, and NASBAs have been developed for the rapid identification of dengue virus to the serotype level with more accuracy (Kuno 1998; Lanciotti et al. 1992; Morita et al. 1991; Seah et al. 1995; Sudiro et al. 1997). Magnitude of amplification can be obtained easily by these PCR-based methods, but they require either high-precision instruments for the amplification or elaborate methods for detection of the amplified products. In addition, these methods are often cumbersome to adapt to routine clinical use, especially in peripheral health care settings and private clinics. Parida et al. (2005) developed RT-LAMP assay for reducing time required for the confirmation of the detection of dengue virus. LAMP assay detects dengue virus within 30 min only (less than 1 h), whereas RT-PCR requires 3–4 h for same detection. Also it was found to be 10- to 100-fold more sensitive than RT-PCR, with a detection limit of 0.1–1.0 PFU of virus.

Varicella (chickenpox) is a common and highly contagious disease in childhood that is caused by primary infection with varicella-zoster virus (VZV). The virus infects the dorsal root ganglia after primary infection, and its reactivation leads to shingles (zoster) in older individuals. In 1995, universal varicella immunization was started in the United States with VZV Oka (vOka strain) vaccine. Molecular analysis of the VZV genome (obtained from vesicular regions) used to discriminate the vOka strains and wild-type strains was based on restriction fragment length polymorphism analysis of the viral genomes from isolated viruses (La Russa et al. 1992). But this method had limited general clinical use because the virus had to be isolated from the vesicular region. PCR methods were used to overcome this problem, PCR which can detect single nucleotide polymorphism (SNP) mutations in open reading frame 38 (ORF38), ORF54, and ORF62 had been developed to distinguish the vOka and wild-type strains (Argaw et al. 2000; Quinlivan et al. 2005). Higashimoto et al. (2008) developed LAMP method to distinguish between the varicella-zoster virus (VZV) vaccine (vOka) strain and wild-type strains. Two single nucleotide polymorphisms (SNPs) (nucleotide [nt] 105705 for VR-1 VZV LAMP and nt 106262 for VR-2 VZV LAMP) located in the open reading frame 62 gene were selected as LAMP targets. The detection limits of both LAMP methods were 100 copies per reaction, which is higher than that of the previously reported VZV LAMP method (Okamoto et al. 2004), and also this method can detect viral DNA directly from swab samples without need of DNA extraction.

Commercial kits have been developed by using LAMP method, and some of them have been
adopted as the officially recommended methods for the routine identification and surveillance of pathogens in Japan (Mori and Notomi 2009).

21.4.2 Plant Viruses

LAMP had been developed for many plant viruses such as tobacco mosaic virus (Liu et al. 2010), banana streak virus (Peng et al. 2012), cauliflower mosaic virus (Fukuta et al. 2004), tomato spotted wilt virus (Fukuta et al. 2004), yellow mosaic virus (Fukuta et al. 2003), potato virus Y (Nie 2005), potato spindle tuber viroid (Tsutsumi et al. 2010), peach latent mosaic viroid (Tsutsumi et al. 2010), wheat yellow mosaic virus (Zhang et al. 2011), necrotic spots virus (Gunimaladevi et al. 2005), and chrysanthemum stunt virus (Fukuta et al. 2005).

The most commonly known peach viroid is peach latent mosaic viroid (PLMVd) which causes a vast majority of natural infections (except leaf symptoms) but long time is required for the onset of symptoms. So a sensitive, accessible, reliable, cost-effective, and fast diagnostic method is in demand to control PLMVd spread and the production of healthy and of high-quality propagation material. Boubourakas et al. (2009) developed an RT-LAMP protocol for the detection of PLMVd with a detection limit of $10^{-5}$. RT-LAMP products were confirmed for the presence of corresponding sequences of the selected target by subjecting a portion of the amplified products to the restriction enzyme analysis (Kubota et al. 2008).

Tsutsumi et al. (2010) developed RT-LAMP for the detection of potato spindle tuber viroid (PSTVd-S). Potato spindle tuber viroid (PSTVd) is a species of the genus Pospiviroid in the family Pospiviroidae that occurs in China (He et al. 1987), Europe, and so on. PSTVd infects potatoes (Solanum tuberosum), tomatoes (Lycopersicon esculentum), and other Solanum spp. (Singh 1973). PSTVd causes stunting, smaller and elongated tubers, and yield losses of potatoes (Pfannenstiel and Slack 1980). The occurrence of PSTVd has not been recorded in Japan, except a temporary occurrence in a greenhouse in Fukushima Prefecture in 2008. As a result of investigation of its cause, it could be thought to originate from imported tomato seeds. Sensitivity for PSTVd-S was determined from extracted DNA of infected potato leaf and serially diluted up to $10^{-4}$ times with sterilized water. It was possible to detect PSTVd from the extracted total nucleic acid diluted $10^{-3}$ in RT-LAMP method and diluted to $10^{-2}$ times in RT-PCR method. RT-LAMP method was ten times higher sensitive than RT-PCR method.

Nie (2005) developed reverse transcription loop-mediated isothermal amplification of DNA for the detection of potato virus Y. Coat protein (CP) gene was selected for designing the primers in such a way that a loop could be formed and elongated during DNA amplification. LAMP assay was found to be 10-fold more sensitive than conventional PCR.

Wheat yellow mosaic as one of the most devastating soilborne disease of winter wheat (Triticum aestivum L.) was first reported in Japan in the 1920s and China in the 1960s (Sawada 1927; Kusume et al. 1997) which spread continually in Japan and China (Wang et al. 1980; Han et al. 2000). A soilborne pathogen, i.e., wheat yellow mosaic virus (WYMV), was the causal agent responsible behind this disease, and it is transmitted by the fungus-like organism polymyxa graminis (Inouye 1969). Several common methods were used to detect WYMV, and ELISA was found as a reliable and suitable method for detecting high-throughput WYMV (Hariri et al. 1996a, b; Geng et al. 2003), but the sensitivity of ELISA might not be sufficiently high to detect low concentrations of WYMV; plus virus-specific antiserum is required for ELISA assay. Zhang et al. (2011) developed RT-LAMP as a rapid detection method for wheat yellow mosaic virus. RT-LAMP could detect WYMV from total RNA diluted up to $10^{-5}$, while RT-PCR detected only up to $10^{-3}$. So, RT-LAMP was 100 times more sensitive than conventional reverse transcriptase-polymerase chain reaction (RT-PCR).

Banana streak virus (BSV) is a significant constraint to banana production and genetic improvement. Therefore, Peng et al. (2012) developed an assay for rapid detection of banana streak virus by loop-mediated isothermal amplification assay
in South China. LAMP assay could detect as low as 1 pg/μl template DNA. Test results of all field samples collected from different regions of South China showed that LAMP is more sensitive than PCR.

21.4.3 Other Animal Viruses

LAMP had been developed for the following viruses. Foot-and-mouth disease virus (Dukes et al. 2006), viral conjunctivitis (Wakabayashi et al. 2004), porcine reproductive and respiratory syndrome virus and porcine circovirus (Rovira et al. 2009), duck virus enteritis (Ji et al. 2009), monkeypox virus (lizuka et al. 2009), infectious hematopoietic necrosis virus (IHNV) (Gunimaladevi et al. 2004), Newcastle disease virus (Pham et al. 2005), and pestiviruses cause diseases in animals such as classical swine fever (CSF) and bovine viral diarrhea/mucosal disease (BVD/MD). Equine arteritis virus (EAV), porcine reproductive and respiratory syndrome virus (PRRSV), lactate dehydrogenase elevating virus (LDV) of mice, and simian hemorrhagic fever virus (SHFV) all belong to the Arteriviridae family of viruses.

Coronaviruses are positive-strand, enveloped RNA viruses. Viruses in this group are important pathogens of mammals and birds. They cause enteric or respiratory tract infections in a variety of animals including humans, livestock, and pets. Severe acute respiratory syndrome (SARS) is caused by a coronavirus called SARS-CoV. CoV-based vectors have potential in vaccine development and for gene therapy (Theil 2007). Severe acute respiratory syndrome (SARS) first spread in Guangdong Province, China in November 2002 (Zhong et al. 2003). SARS is diagnosed by two major approaches: first, detection of antibodies against SARS-CoV was a sensitive and specific diagnostic approach, but seroconversion can be detected only around after day 10 of illness (Peiris et al. 2003a). In contrast, PCR-based tests have been shown to be useful for early SARS diagnosis (Berger et al. 2004). Quantitative PCR approaches are a powerful tool for identifying SARS-CoV early after disease onset (Drosten et al. 2003; Poon et al. 2003; Ng et al. 2003; Grant et al. 2003). Because of the requirement of sophisticated instrumentation and expensive reagents, these rapid molecular tests might not be the method of choice in field situations. The detection limit of the assay was 10 copies/reaction, and positive signals were consistently observed in reactions containing ~50 copies of the target sequence. Quantitative PCR method was used for SARS diagnosis (Poon et al. 2003). Thai et al. (2004) developed a one-step, single-tube, accelerated, real-time, quantitative RT-LAMP assay for the early and rapid diagnosis of SARS-CoV. The sensitivity and specificity of RT-LAMP assay for detecting viral RNA in clinical specimens with regard to RT-PCR were 100 and 87 %, respectively. The RT-LAMP assay was found to be 100-fold more sensitive than RT-PCR, with a detection limit of 0.01 PFU in clinical samples. In addition, by using a real-time RT-LAMP assay, the quantitation of virus concentration in the clinical sample is possible, which will indicate the early stage of the virus infection as well as potential source transmitters. However, the detection rates for SARS in the LAMP assay are similar to those with our conventional PCR-based assays (Chan et al. 2004). Rapid detection of the severe acute respiratory syndrome (SARS) coronavirus by a loop-mediated isothermal amplification assay was developed by Poon et al. (2004). The detection limit of the assay was 10 copies/reaction reactions containing ~50 copies of the target sequence. Viral load peaks in the second week of infection in SARS patients due to which its detection is difficult. LAMP assay is an inexpensive and accurate alternative method for detection of virus at the end of first week of infection (Peiris et al. 2003b).

Foot-and-mouth disease (FMD) causes severe economic losses and affected both national and international trade within the livestock and animal products (King et al. 2006). So, it was quite urgent and necessary to control causative virus responsible behind this veterinary infection. Chen et al. (2011) successfully detected foot-and-mouth disease viral RNA by reverse transcription loop-mediated isothermal amplification method with the detection
limit of 10 copies, whereas RT-PCR detected 100 copies per reaction.

African swine fever virus causes a viral hemorrhagic fever with high mortality rates in pigs but persistently infects its natural hosts, warthogs, bushpigs, and soft ticks, with no disease signs (Viruses of Animals, George Durrell http://EzineArticles.com/?Expert=George_Durrell). Pseudorabies virus causes Aujeszky’s disease in pigs and is extensively studied as a model for basic processes during lytic herpesvirus infection. Bovine herpesvirus-1 causes bovine infectious rhinotracheitis and pustular vulvovaginitis. The avian infectious laryngotracheitis virus is phylogenetically different from these two viruses. A large variety of influenza A viruses infect wild aquatic birds. Major economic losses in poultry production are due to highly pathogenic avian influenza virus (HPAIV) which also threatens human health. For rapid AIV detection, new assays would be designed that can be carried out with a minimum of specific equipment as compared to conventional methods. Although RT-LAMP was markedly more vulnerable to reduce the detection limits because of strain-specific sequence variation than subtype-specific real-time RT-PCR. Because of simplicity, LAMP proves to be a promising technology for AIV diagnosis (Notomi et al. 2000; Parida et al. 2008). Real-time RT-PCR also represents the gold standard for AIV RNA detection as it is considerably more sensitive than conventional RT-PCR (Pasick 2008). All RNA samples tested specifically positive in the real-time RT-LAMP were also assayed for sensitivity and compared with real-time RT-PCR. All AIV-targeted RT-LAMP assays developed so far have been compared with conventional RT-PCR, and sensitivity was calculated on the basis of infectivity, not taking into account the amount of RNA copies (Chen et al. 2008; Imai et al. 2006, 2007; Jayawardena et al. 2007). Chen et al. (2010) diagnosed H9 avian influenza virus by using reverse transcription loop-mediated isothermal amplification with a detection limit of ten copies per reaction, and no cross-reactivity was observed from the samples of other related viruses including H5N1, H3N2 subtype of AIV, and Newcastle disease virus. Again in 2011, Nagatani et al. detected RNA of influenza virus by reverse transcription LAMP using a USB-powered portable potentiostat.

Xie et al. (2010) developed an assay for rapid detection of infectious laryngotracheitis virus isolates. LAMP assay was found ten-fold more sensitive than the routine PCR assay, with a detection limit of 46 copies per reaction.

Gunimaladevi et al. (2004) developed LAMP method for detection of infectious hematopoietic necrosis virus (IHNV) in rainbow trout (Oncorhynchus mykiss) with a ten-fold higher sensitivity compared with conventional nested PCR.

Porcine circoviruses (PCV) are the smallest autonomously replicating viruses in eukaryotic cells. Among known two types of PCV, porcine circovirus type 1 (PCV1) does not cause disease, but porcine circovirus type 2 (PCV2) causes a new emerging and multifactorial disease in swine known as postweaning multisystemic wasting syndrome (PMWS) (Bolin et al. 2001) and porcine dermatitis and nephropathy syndrome (PDNS). Porcine circovirus type 2 (PCV2) syndrome is responsible for great losses to the pig industry. PCV2 is a non-enveloped, circular, single-stranded DNA virus. Presence of PCR inhibitors (including organic and inorganic substances such as detergents, antibiotics, phenolic compounds, enzymes, polysaccharides, fats, proteins, and salts) in the analysis of real biological samples limits the usefulness of PCR. These inhibitors reduce the amplification efficiency (Darwich et al. 2002; Guo et al. 2010). PCV2 was detected by reverse transcription LAMP using membrane protein M gene for primers with a detection limit ten fold greater than conventional PCR (Chen et al. 2009). Chen et al. (2008) detected PCV2 by LAMP by using only four primers, while Zhao et al. (2011) detected same virus by efficient LAMP assay using six primers including two loop primers for detection of target ORF2 gene in PCV2 (which encodes Rep protein that is involved in virus replication) with a detection limit of 10 copies, whereas the limit by conventional PCR was 1,000 copies. LAMP assay had advantages in specificity, selectivity, and rapidity over other nucleic acid amplification methods (Mori et al. 2006). LAMP had been further advanced by using
forward loop primers (Nagamine et al. 2002). The LAMP assay developed had a detection limit of ten copies for PCV2, which was 100-fold more sensitive than conventional PCR.

Rovira et al. (2009) developed reverse transcription LAMP for porcine reproductive and respiratory syndrome virus detection. The limit of detection ranged between $10^{-2}$ and $10^{-4}$ (50% tissue culture infective) dose/ml.

Swine transmissible gastroenteritis coronavirus (TGEV) is a kind of single-stranded RNA virus, which produces villous atrophy and enteritis, leading to the serious financial loss to the whole pig industry. The traditional detection methods including virus isolation, virus immunodiagnostic assays, and PCR tests have shortcomings such as precise instruments requirement, demand of elaborate result analysis, high cost, and long detection time, which prevent these methods from being widely used (Reynolds and Garwes 1979; Rodak et al. 2005; Denac et al. 1997; Paton et al. 1997). TGEV can be detected by using loop-mediated isothermal amplification with the detection limit of about 10 pg RNA which is ten times more sensitive than that of PCR and having no cross-reaction with other viruses (Chen et al. 2010) and found that the most conserved fragment of 187 bp was found in the nucleocapsid protein gene which showed highly homology among different TGEV strains/isolates (more than 97%) and low homology among other similar viruses (less than 52.5%). LAMP could not only qualitatively detect the TGEV but also quantitatively analyze the virus. It was concluded that real-time fluorescence LAMP for quantitatively detection of TGEV was established by adding 1X SYBR Green I in the LAMP reaction.

Duck virus enteritis is a serious disease among farmed and free-living ducks (Anatidae) and a constant threat to the commercial duck industry in China. Ji et al. (2009) developed LAMP method by using set of four specific primers designed to recognize six distinct genomic sequences of UL6 protein from duck plague virus (DPV). The optimum reaction temperature and time were verified to be 61.5 °C and 60 min, respectively. Marek’s disease viral genome in chicken feathers can be easily detected by LAMP with detection limit of ten copies of the MEQ gene in the MD viral genome along with ten times higher sensitivity than the traditional PCR methods (Angamuthu et al. 2012).

By using 12 dogs and 2 cats, Boldbaatar et al. (2009) developed reverse transcription LAMP for detection of rabies virus with detection limit of $10^3$ copies of viral RNA (corresponding to approximately 5 fg of RNA).

Nimitphak et al. (2010) developed a rapid and sensitive detection of *Penaeus monodon nucleopolyhedrovirus* (*PemoNPV*) by loop-mediated isothermal amplification combined with a lateral-flow dipstick. With a DNA template extracted from PemoNPV-infected shrimp, the LAMP-LFD detection limit was 0.1 pg, whereas one-step PCR and nested PCR followed with gel electrophoresis was 1 pg. Fang et al. (2011) developed for predicting viruses accurately by a multiplex microfluidic loop-mediated isothermal amplification chip. Multiplex gene assay is a valuable molecular tool not only in academic science but also in clinical diagnostics. An octopus-like multiplex microfluidic loop-mediated isothermal amplification (µLAMP) assay proved a robust approach for predicting viruses for the rapid analysis of multiple genes in the point-of-care format. It has ability of analyzing multiple genes qualitatively and quantitatively as it is highly specific, operationally simple, and cost/time-effective with the detection limit of less than 10 copies/µl in 2 µl quantities of sample within 0.5 h. A multiplex microfluidic LAMP chip for differentiating three human influenza A substrains and identifying eight important swine viruses had been successfully developed.

In 2008, Blomstrom et al. developed single-step reverse transcriptase LAMP assay for simple and rapid detection of swine vesicular disease virus with a detection limit of 50 viral RNA copies per µl.

Rift Valley fever virus (RVFV) is a zoonotic mosquito-borne virus with tripartite negative-strand RNA genome composed of a large segment (L), encoding the viral transcriptase, a medium segment (M), coding for the two external glycoproteins (GN and GC), and an S segment, which codes for the nucleocapsid protein (N) and a nonstructural...
protein (NSs) (Schmaljohn 1996). The virus causes explosive outbreaks in animals and humans (Durand et al. 2003) and has been observed in Egypt, in Mauritania, and more recently in the Arabian Peninsula (Bird et al. 2007). Domestic animals are sensitive to RVFV infection and amplify the virus to high titers (Zeller and Bouloy 2000). Among cattle, sheep, goats, pigs, and camels, infection causes fever and anorexia. Peyrefitte et al. (2008) also developed a real-time reverse transcription LAMP for rapid detection of Rift Valley fever virus. The assay is highly sensitive and comparable to real-time RT-PCR, with a detection limit of ~10 RNA copies per assay.

Newcastle disease is a highly contagious viral infection of poultry caused by a paramyxovirus called avian paramyxovirus type 1 (APMV-1), one of the nine serotypes of the virus identified (Alexander and Manvell 1997). Pham et al. (2005) developed loop-mediated isothermal amplification for rapid detection of Newcastle disease virus. The detection limit of both methods, i.e., PCR as well as LAMP, was 0.5 pg or 9 × 10^6 copies/reaction determined by using a serial ten-fold dilution of the plasmids.

Parida et al. (2004) developed real-time reverse transcription LAMP method for rapid detection of West Nile virus by using envelope gene of West Nile (WN) virus. Suebsing et al. (2011) developed reverse transcription LAMP method for detection of infectious hematopoietic necrosis virus in Oncorhynchus keta. The limit of detection was 0.01 fg of RNA extracted from IHNV-infected CHSE-214 cells, compared with 1.0 fg for nested RT-PCR. Ren and Li (2011) developed reverse transcription loop-mediated isothermal amplification for rapid detection of porcine epidemic diarrhea virus. Six primers were designed to amplify the nucleocapsid (N) gene of PEDV. The optimal reaction condition for RT-LAMP amplifying PEDV N gene was achieved at 63 °C for 50 min. It was found that RT-LAMP assay was more sensitive than gel-based RT-PCR and enzyme-linked immunosorbent assay. It was capable of detecting PEDV from clinical samples and differentiating PEDV from porcine transmissible gastroenteritis virus, porcine rotavirus, porcine pseudorabies virus, porcine reproductive and respiratory syndrome virus, and avian infectious bronchitis virus. Taura syndrome virus (TSV) was first discovered in Ecuador in 1992 (Jimenez 1992). Kiatpathomchai et al. (2007) developed a rapid and sensitive detection of Taura syndrome virus by reverse transcription loop-mediated isothermal amplification. The detection of TSV using RT-LAMP was ten times more sensitive than the RT-PCR, but less sensitive than nested RT-PCR. Sappat et al. (2011) detected the shrimp Taura syndrome virus by loop-mediated isothermal amplification using a designed portable multichannel turbidimeter. When using the same TSV-RNA template sets, the results revealed the same detection limit of 10^-6 dilution for RT-LAMP when performed using a commercial heating block (Major Science, Taiwan) and the designed turbidimeter. When RT-LAMP reactions were carried out at 63 °C for 30 min using 2 μl of the ten-fold serially diluted RNA template extracted from TSV-infected shrimp, the turbidity measurement was able to detect 100 fg of total RNA. This detection limit was equivalent to that of RT-LAMP-AGE and nested RT-PCR. When the RNA in vitro transcript was used as the template, the detection limit by both of turbidity measurement and AGE was ten copies. A prior study indicated that the LAMP method was capable of amplification starting with only six copies of template (Nagamine et al. 2002).

Xie et al. (2011) rapid detection of group I avian adenoviruses by a loop-mediated isothermal amplification. In total, 72 out of 184 cloacal swab samples from poultry were identified as positive by LAMP, whereas 45 out of 184 were identified as positive by conventional PCR test. Liu (2011) reverse transcription loop-mediated isothermal amplification for the rapid detection of infectious bronchitis virus. Infectious bronchitis virus (IBV) is a major cause of disease in domestic fowl and causes an acute, highly contagious disease of the respiratory tracts and sometimes urogenital tracts (King and Cavanagh 1991). The result indicated detection limit of IBV RT-LAMP was 10 copies/
tube. In addition, the reaction time of RT-LAMP method is 45 min, which is more rapid than conventional RT-PCR, and the reaction only needs a laboratory water bath. Kiatpathomchai et al. (2007) detected RT-TSV RNA in *P. vannamei* (collected from shrimp farms) by LAMP assay, and the sensitivity of RT-LAMP appears to be ten times more than RT-PCR. Wang et al. (2011) detected infectious bursal disease virus (IBDV) in one simple step by reverse transcription loop-mediated isothermal amplification (RT-LAMP) and further identified the very virulent strain from non-vvIBDVs with a simply post-amplification restriction enzyme analysis. A set of two inner, two outer, and two loop primers were designed on the basis of sequence analysis to target the VP5 gene and showed great specificity with no cross-reaction to the other common avian pathogens. Detection limit was determined by both color change inspection and agarose gel electrophoresis was 28 copies viral RNA, which was almost as sensitive as a real-time RT-PCR previously developed.

Last year in 2012, Foord et al. developed hendra virus detection using loop-mediated isothermal amplification. Hendra virus (HeV) is a zoonotic paramyxovirus endemic in Australian Pteropus bats (fruit bats or flying foxes). Although bats appear to be unaffected by the virus, HeV can spread from fruit bats to horses, causing severe disease. Human infection results from close contact with the blood, body fluids, and tissues of infected horses. HeV is a biosecurity level 4 (BSL-4) pathogens, with high fatality rate in humans and horses. Current assays for HeV detection require complex instrumentation and are generally time-consuming. Analytical sensitivity and specificity of the HeV-LAMP assay was equal to a TaqMan assay developed previously.

The viruses for which LAMP has not been developed till now are as follows: equine artemis virus, simian hemorrhagic fever virus, tick-borne encephalitis virus, bornavirus, infectious bovine rhinotracheitis virus, feline herpesvirus, bovine respiratory syncytial virus, bovine rhinotracheitis virus, equine anemia virus, cowpox virus, elephant endotheliotropic herpesvirus, turkeypox virus, myxomatosis virus, menangle virus, rabbit hemorrhagic disease virus, and rabbit hemorrhagic virus.

### 21.5 Conclusions

Compared with RT-PCR and real-time PCR, LAMP has the advantages of reaction simplicity and detection sensitivity. The higher sensitivity and specificity of the LAMP reaction is attributed to continuous amplification under isothermal conditions employing six primers recognizing eight distinct regions of the target. Additionally, the higher amplification efficiency of the LAMP reaction yields large amounts of by-product (pyrophosphate ion) leading to a white precipitate of magnesium pyrophosphate in the reaction mixture. Since the increase in turbidity of the reaction mixture—according to the production of precipitate—correlates with the amount of DNA synthesized, real-time monitoring of the LAMP reaction can be achieved by real-time measurement of turbidity. The LAMP assay has emerged as a powerful gene amplification tool for the rapid identification of microbial infections and is being increasingly used by various investigators for the rapid detection and typing of emerging viruses, including WNV, severe acute respiratory syndrome (SARS), dengue, and JEV.

### 21.6 Future Prospects

Some other viruses for which LAMP has not been developed till now are as follows: tomato bushy stunt virus, peanut stunt virus, peony ring spot virus, psoriasis A virus, bean common mosaic virus, lettuce mosaic virus, fanleaf disease virus, wheat streak mosaic virus, bud blight virus, rose mosaic virus, zucchini yellow mosaic virus, cankers virus, alfalfa mosaic virus, bean yellow mosaic virus, tobacco etch virus, wound tumor virus, pea enation mosaic virus, quirking virus (on datura), sugarcane mosaic virus, etc.
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