Introduction
Infectious diseases present challenges to both early diagnosis and adequate management. Novel nucleic acids amplification methods are developed in order to meet these challenges. The polymerase chain reaction (PCR) is among the most widely used but alternatives are present, such as nucleic acid sequence-based amplification, NASBA (1), self-sustained sequence replication, 3SR (2) and strand displacement amplification, SDA (14). They are all based on their own principle to induce a cycle-to-cycle transition. However, all of them altogether with PCR have their disadvantages. PCR requires a specific equipment, 3SR and NASBA have problems with their specificity and range of application, and SDA is difficult to apply under regular conditions. Recently, a new technique for detection of genomic sequences was introduced, termed loop-mediated isothermal amplification (LAMP). The method was then applied for several important human pathogens. It has been proven to be both cost and time effective, making it possible for the physician to have a reliable and fast laboratory result, as $10^9$ specific amplicons are produced in less than an hour (9).

The following material describes the principle of LAMP and its advantages. Also applications of this technique for detection of some widespread viral pathogens have been discussed.

Principle of LAMP
The method loop-mediated amplification has been developed by Eiken Chemical Co., Japan (9). It is based on the use of a minimal set of four oligonucleotides (primers) that are specifically designed to recognize six precise regions from a targeted genomic sequence (Fig. 1). Two of the oligonucleotides that are termed forward and backward inner primers (FIP and BIP) each possess two sequences. Unlike primers that are used by other techniques, such as the PCR oligos, FIP and BIP are complementary to sequences from both strands of the targeted DNA that have close location to each other (designated as F1 and F2c for FIP and B1 and B2c for BIP, respectively). The other two oligonucleotides, termed outer primers (F3 and B3) are like ordinary PCR primers and are complementary to the sequences F3c and B3c on Fig. 1. Initially, all four primers are necessary for formation of a specific double-stranded structure with loops at each end (Fig. 2).

**Fig. 1.** Schematic presentation of a LAMP primer set targeted to a particular genomic region

**Fig. 2.** Dumb-bell form produced in the initial stages of LAMP
several steps can be performed simultaneously speeding up the process. The synthesis of DNA is done by a DNA polymerase such as Bst that only contains 5’ → 3’ polymerase activity, but lacks 5’ → 3’ exonuclease activity. Therefore a strand displacement process can be ensured. As these polymerases are not thermostable they cannot be used in a PCR reaction, but the LAMP is an isothermal cycling technique that is held under optimal temperature conditions of approximately 65°C. The reaction produces a mixture of stem-loop and multi-loop cauliflower-like structures that are all constructed from multiple repeats of only the targeted genomic sequence.

Particularly LAMP can be used for detection of any genomic sequence. RNA pathogens can be easily identified by just adding an initial reverse transcriptase (RT) step (9).

Another improvement of the method includes the development of additional primer pair (to a total of six oligonucleotides) with loops already synthesized in the designed primers. Thus, the cycling stage starts earlier and both the sensitivity and specificity of the reaction are improved even further (7).

Detection methods for LAMP products
Magnesium pyrophosphate, a byproduct of the amplification process, is produced in proportional amounts to the amplified product. LAMP is highly efficient and large amounts of end-products are synthesized, altogether with large amounts of pyrophosphate. The white turbidity attributed to it may be visually observed. Therefore, the presence of turbidity indicates the formation of the targeted genomic region.

Visual detection can also be achieved by adding an intercalating dye, most often SYBR Green I, to the final reaction mixture. When UV light is applied the presence of specific product would be recognized by green fluorescence.

A real-time follow up of the reaction can be achieved as turbidity can be measured with an instrument called turbidimeter. This allows for quantitative detection of pathogens.

If an agarose gel electrophoresis of the LAMP product is performed, bands with various sizes will be visualized at regular intervals. Additionally, restriction analysis alone or followed by sequencing reactions can be performed for verification of new protocols that are tested for the first time.

Advantages of LAMP
Under optimal conditions the LAMP reaction produces a tremendous amount of a targeted sequence ($10^9$-$10^{10}$ copies) for less than an hour (8). The process is isothermal and can be carried out in a simple thermostat or water bath.

The method is comparable to PCR in terms of sensitivity, but is less affected by presence of non-targeted DNA and inhibitory molecules. Some researchers even report specific amplification with LAMP without prior extraction procedure, by directly adding reaction mixture to swab specimens or sera (3), (15).

LAMP is highly specific as six separate genomic regions in the initial stage and four in the later steps, need to be recognized in order that the reaction is carried out. Therefore, a successful reaction would indicate the presence of the targeted sequence.

Applications of LAMP for detection of human viruses
Both DNA and RNA viruses can be detected by LAMP and diagnosis of several important emerging and re-emerging diseases by this technique has already been reported. Ihira et al. (3) and Kuhara et al. (5) in 2007 developed and tested LAMP for HHV-6 and HHV-8. The method was found to successfully identify as low as 10 copies per reaction. (15) used LAMP to detect parvovirus B19 and suggest the new technique to replace other methods like serological analyses and PCR, because the authors found them either time consuming or more expensive, respectively.

RT-LAMP was reported to be useful for detection of viral pathogens that cause hemorrhagic fevers such as ebolavirus (6). The researchers report the sensitivity of the system to be 20 copies per test tube and identification could be achieved in 30 minutes. Primer sets for LAMP have also been developed for detection of each of 4 Dengue serotypes (10).

Another research group reports the use of LAMP for detection of the coronavirus responsible for the development of the Severe Acute Respiratory Syndrome, SARS (8).

The list of successful protocols for LAMP detection also includes pathogens like West Nile virus (11), Chikungunya virus (12), Japanese encephalitis virus (13), Avian flu H5N1 virus (4) and mumps virus (16).

Conclusions
The presented loop-mediated isothermal amplification method-LAMP is reported to be simplified and efficient technique for detection of viral pathogens. The advantages of LAMP make it a suitable field test tool for diagnosis of emerging and re-emerging diseases. As it is relatively cheap and reliable most probably LAMP will be more widely integrated into routine use of molecular techniques.

REFERENCES

BIOTECHNOL. & BIOTECHNOL. EQ. 24/2010/1