# Loop Mediated Isothermal Amplification Of Dna

Nucleic acid diagnostic tests can complement existing tools to improve the diagnosis of diseases. However, the requirement of laboratory infrastructure limits their use in developing countries as routine tests. Isothermal amplification techniques such as loop-mediated isothermal amplification bridge such challenges because they require simpler infrastructure. In this study, we evaluated a commercial H5N1 Avian influenza virus (AIV) detection kit using isothermal amplification with Bsm DNA polymerase. We also developed a one-step RT-LAMP assay using two strand displacing DNA polymerases for the detection of two zoonotic viruses, avian influenza A virus and lyssaviruses. During the evaluation of the commercial H5N1 detection kit, A/H9N2, two A/H7N3 isolates and Influenza B were also detected indicating non-specific detection. After optimising reaction temperature and time only the Influenza B was non-specifically detected. In addition, a reverse-transcription LAMP assay was developed focusing on the matrix gene of avian influenza A virus. The detection limit and specificity of the assay was tested using serially diluted in vitro transcribed RNA and the different subtypes of influenza virus using optimal reaction conditions. Restriction enzyme digestion and nucleotide sequencing was used to confirm the identity of the amplified RT-LAMP product. Two detection methods, agarose gel electrophoresis and realtime fluorescence using a fluorescence reader, ESE-Quant tube scanner (ESE Gmbh, Stockach, Germany), were evaluated. The sensitivity of these two detection methods was similar; however, the real-time monitoring of amplification is more suitable for field application of the RT-LAMP assays. A reverse-transcription loop-mediated isothermal amplification assay was also developed for the specific detection of rabies and other lyssaviruses. The assays used specifically designed primers to target the partial nucleoprotein (N) gene and were able to amplify all 12 lyssavirus species; representing a wide diversity of lyssaviruses present in Africa. RT-LAMP reaction was confirmed by restriction enzyme analysis and sequencing. The use of melting curve analysis was also attempted. The assay was about 1000 times more sensitive compared to the RTPCR assay. The RT-LAMP assays described here have great potential as a diagnostic tool as well as an on-site molecular tool especially in resource-limited settings.

In the recent years, massive losses in shrimp production were observed due to acute hepatopancreatic necrosis disease (AHPND) related outbreaks. There are currently no available cure for this disease and early detection methods remain as the primary way to combat effects of the disease. Since AHPND is a relatively new disease in shrimps, available detection methods remain limited to histopathological analyses and polymerase chain reaction (PCR). Another method explored to detect the disease is Loop-mediated isothermal amplification (LAMP) which is known for specific and fast diagnosis. Here, the development of alternative LAMP primers specifically designed for the Philippine strain of Vibrio

parahaemolyticus causing AHPND was discussed. The sensitivity of the established LAMP protocol was found to be at 4ug/mL of bacterial DNA. At the same time, it exhibited high specificity to AHPND-causing V. parahaemolyticus. Using the locally designed LAMP primers AHPND was detected in 78% of samples from Luzon, 46% in Visayas and 71% in Mindanao . In comparison with PCR, LAMP was able to detect 54% of samples positive for AHPND while only 40% tested positive in PCR. In summary, AHPND is still a crippling disease in terms of shrimp production in the Philippines and that the developed AHPND-LAMP primers in this study could be of great alternative in the detection of the disease.
Development of Loop-mediated Isothermal Amplification (LAMP) Method Using a Simple Turbidimeter for Detection of Infectious Myonecrosis Virus (IMNV) and Macrobrachium Rosenbergii Nodavirus (MrNV)Rapid and Simple Colorimetric Loop-mediated Isothermal Amplification (LAMP) Assay Fpr the Detection of Bovine Alphaherpesvirus 1Development of Loop-Mediated Isothermal Amplification Assay for Rapid Diagnosis of TuberculosisLoop-Mediated Isothermal Amplification for the Detection of HLA B*5801 Associated Allopurinol HypersensitivityDevelopment of Loop-mediated Isothermal Amplification Assay for Rapid Diagnosis of TuberculosisEntwicklung und Evaluation von Loop mediated isothermal amplification (LAMP)-basierter Paratyphus-DiagnostikEvaluation of Loop-mediated Isothermal Amplification for Detection of Salmonella Specific GeneLoop-Mediated Isothermal Amplification (Lamp) for TuberculosisLAP Lambert Academic Publishing
The WHO End TB Strategy calls for the early diagnosis of TB and universal drug susceptibility testing (DST), highlighting the critical role of laboratories for rapidly and accurately detecting TB and drug resistance. Molecular assays based on nucleic acid amplification techniques such as polymerase chain reaction (PCR) have been developed for rapid TB diagnosis and are being implemented in developing countries. A commercial molecular assay Loopamp MTBC Detection Kit based on loop-mediated isothermal amplification was developed by Eiken Chemical Company Ltd (Tokyo, Japan) for the detection of Mycobacterium tuberculosis complex (TB-LAMP). TB-LAMP is a manual assay that requires less than one hour to perform and can be read with the naked eye under ultra violet light. Following review of the latest evidence, WHO recommends that TB-LAMP can be used as a replacement for microscopy for the diagnosis of pulmonary TB in adults with signs and symptoms of TB. It can also be considered as a follow-on test to microscopy in adults with signs and symptoms of pulmonary TB, especially when further testing of sputum smear-negative specimens is necessary.
"The IDH1 mutation is an important diagnostic and prognostic biomarker used to characterize glioblastoma (GBM). Patients harboring the IDH1 mutation have improved overall survival following maximal resection. Knowledge of the IDH1 mutation status allows the surgeon to modify the surgical plan; however, no existing molecular test can provide this information intraoperatively. We designed a novel colorimetric peptide nucleic acid loop-mediated isothermal

amplification (PNA-LAMP) method that rapidly detects the IDH1 R132H mutation in GBM. PNA-LAMP amplifies target DNA under isothermal conditions with high specificity and speed. The PNA prevents amplification of wild-type IDH1 DNA, while allowing amplification of the R132H variant if present. We used a pH-sensitive colorimetric detection method for visual determination of amplification in under one hour. Characterization of the assay was performed with plasmid DNA containing the IDH1 wild-type and R132H variant sequences.Amplification was confirmed using gel electrophoresis, and this analysis suggests that the assay is more sensitive than Sanger sequencing - the gold standard for IDH1 mutation identification. This study is the first to attempt to develop a colorimetric LAMP assay for GBM tumor characterization, and only the third application of the PNA-LAMP method to detect acquired mutations in cancer. This novel molecular assay is a simple, specific, and rapid way to identify the presence of the IDH1 R132H variant."--Abstract.

Tuberculosis (TB) is a global health problem and manifests in severe disease. Rapid and accurate diagnosis of symptomatic patients is a cornerstone of global tuberculosis control strategies. Difficulties in current case finding tools in disease endemic countries have made the situation more complicated to ensure access to good diagnostics at all health service levels leaving many patients undiagnosed. New nucleic acid amplification technique called Loop-mediated isothermal amplification (LAMP) has been developed, in which reagents react under isothermal condition with high specificity, efficiency and rapidity for quick detection of Mycobacterium tuberculosis complex (MTBC) in sputum samples of suspected pulmonary tuberculosis (PTB) patients. The present study aims to evaluate the efficiency of LAMP in detecting MTBC in sputum samples of suspected PTB patients under Nepalese settings.

Shiga toxin-producing Escherichia coli (STEC) is a worldwide health concern. To detect STEC, a loop mediated isothermal amplification reaction (LAMP) was optimized to detect shiga toxin genes. LAMP's performance was compared to conventional and real-time (RT) PCR using two product detection methods. All three DNA amplification methods produced similar results. LAMP performed well when tested with randomly selected stool samples. LAMP, with agarose gel detection, showed a sensitivity of 90%/100%, specificity of 95%/99%, a positive predictive value of 56%/89% and a negative predictive value of 99%/100% for stx1 and stx2 respectively. Sybr Green 1 detection had a sensitivity of 100%/100%, specificity of 93%/77%, positive predictive values of 76/39% and negative predictive values of 100/100% for stx1 and stx2 respectively. Per 10 tests LAMP costs approximately $45, when using a rapid lysis DNA extraction and agarose gel electrophoresis product detection. LAMP could be implemented in laboratories without dedicated molecular biology facilities.

Reverse-transcription-loop-mediated isothermal amplification (RT-LAMP) has frequently been proposed as an enabling technology for simplified diagnostic tests for RNA viruses. However, common detection techniques used for LAMP and RT-LAMP have drawbacks, including poor discrimination capability, inability to multiplex targets, high rates of false positives, and (in some cases) the requirement of opening reaction tubes postamplification. Here, we present a simple technique that allows closed-tube, target-

specific detection, based on inclusion of a dye-labeled primer that is incorporated into a target-specific amplicon if the target is present. A short, complementary quencher hybridizes to unincorporated primer upon cooling down at the end of the reaction, thereby quenching fluorescence of any unincorporated primer. Our technique, which we term QUASR (for quenching of unincorporated amplification signal reporters, read "quasar"), does not significantly reduce the amplification efficiency or sensitivity of RT-LAMP. Equipped with a simple LED excitation source and a colored plastic gel filter, the naked eye or a camera can easily discriminate between positive and negative QUASR reactions, which produce a difference in signal of approximately 10:1 without background subtraction. We demonstrate that QUASR detection is compatible with complex sample matrices such as human blood, using a novel LAMP primer set for bacteriophage MS2 (a model RNA virus particle). As a result, we demonstrate single-tube duplex detection of West Nile virus (WNV) and chikungunya virus (CHIKV) RNA.

Influenza A infection is a major public health problem world wide. Four major pandemics in the past century including pandemic H1N1 2009 were caused by influenza A viruses. Different approaches of diagnosis assays have been developed to detect influenza A viruses. However, some techniques such as viral isolation, immunofluorescence assay (IFA) and other molecular assays including RT-PCR and real time RT-PCT have limitation to apply in mobile surveillance and unequipped laboratories in developing countries. In this study, one step reverse transcription loop-mediated isothermal amplification was developed as a rapid, sensitive to detect influenza A viruses. To reach the overall goal, a lamp primer set was designed by PrimerExpoler V4 and developed for a sensitive and specific amplification. The optimal amplification reaction is 63oC for 60 minutes then followed by 80oC for ten minutes. The developed assay is ten times more sensitive than conventional RT-PCR and comparable as real time RT-PCR. It is also highly specific for influenza viruses of different hosts. The colorimetric assay of LAMP products is also sensitive as gel electrophoresis. This developed one step RT-LAMP reveals comparable sensitive to detect the Influenza A viruses in both cloacal and tracheal samples. This method is also an easy to use technique and suitable for field surveillance and screening.

RT-PCR is commonly used for the detection of Bcr-Abl fusion transcripts in patients diagnosed with chronic myelogenous leukemia, CML. Two fusion transcripts predominate in CML, Br-Abl e13a2 and e14a2. They have developed reverse transcriptase isothermal loop-mediated amplification (RT-LAMP) assays to detect these two fusion transcripts along with the normal Bcr transcript.

Acute and chronic sleeping sickness are fatal neglected tropical diseases caused by Trypanosoma brucei rhodesiense and Trypanosoma brucei gambiense respectively (members of the sub-genus Trypanozoon). Accurate diagnostics are needed to guide treatment since the symptoms of disease are non-specific and the drugs that are used for treatment are too toxic to be administered to unconfirmed cases. Tests need to be simple enough to confirm clinical diagnosis of sleeping sickness in poorly-resourced, peripheral health centres and for use as epidemiological tools to detect T. b. rhodesiense in the zoonotic reservoirs of infection. This study focuses upon LAMP (loop-mediated isothermal

amplification) as a novel diagnostic for sleeping sickness that may serve to bridge the gap between the need for sensitive, specific molecular diagnostics on the one hand and 'field-friendly' diagnostics on the other. Here, two previously published LAMP assays for Trypanozoons were compared to classic PCR based methods for the diagnosis of Trypanozoon infection status in 428 cattle blood samples. The results did not support the use of LAMP as an improved system for surveillance of T. b. rhodesiense in the zoonotic cattle reservoir. T. b. rhodesiense and T. b. gambiense subspecies specific LAMP assays were evaluated against traditional reference subspecies specific PCR tests, using DNA purified from 86 cryopreserved trypanosome isolates. Novel LAMP assays for these subspecies were also designed and evaluated. Both the published and novel assays for T. b. rhodesiense (targeting different regions of the SRA gene) were sensitive, specific and reliable when applied to purified DNAs, but were less consistent on field samples. The novel T. b. gambiense LAMP (targeting TgsGP) was sensitive and specific but this was not the case for the published LAMP assay (targeting the 5.8S rRNA gene). However reliability may be less than optimal for LAMP TgsGP. Finally, simple endpoint readout methods for LAMP were evaluated. The colour change reagent hydroxynaphthol blue was identified as the best currently available method taking cost, ease of use and reliability into consideration. In 2009 the number of reported sleeping sickness cases fell below 10,000 for the first time in 50 years. Improved LAMP diagnostics could facilitate the diagnosis of sleeping sickness and support the continued fight against this neglected, but deadly disease. Loop-mediated isothermal amplification (LAMP) assay is a recently developed diagnostic technique used for the presence/absence test of pathogens. Unlike traditional PCR methods, LAMP creates a single-strand loop structure after recognition, amplifies under iso-temperature, and results in a cauliflower-like PCR product. This implies that there is a possibility to grow DNA aggregates by fixing template DNA and LAMP mixture in a polyacrylamide gel, and determine bacterial concentrations by enumerating DNA aggregate forming units (DAFU). Microbial enumeration in wastewater biosolids by Real-Time PCR is limited by the abundance of background DNA that only small amount of template DNA can be used. The aggregate forming unit technique allows a much larger template DNA for analysis and thus lowers the detection limit. The objective of this research, therefore, is to develop a DAFU technique to enumerate Escherichia coli (E. coli), a common indicator for wastewater. Serial dilutions of E. coli DNA were analyzed by both conventional LAMP and the developed DAFU technique with malB gene as the target. The LAMP-DAFU contains the same amount of DNA template, same final concentrations of all LAMP reagents, except with the addition of polyacrylamide, tetramethylethylenediamine (TEMED), and ammonium persulfate (APS). The result of conventional LAMP versus LAMP-DAFU revealed that the latter technique was able to detect DNA at a lower concentration. This implies that the developed method not only can be used to enumerate E. coli but also can provide better detection sensitivity. Furthermore,

quantification of E. coli DNA copies with digital PCR showed the presence of 6.9 x 105 DNA copies/[mu]l which is comparable to the result obtained by LAMP-DAFU detection at 6.0 x 105 DAFU/[mu]l. Thus, the results showed the developed method has good accuracy for E. coli enumeration. When LAMP-DAFU was performed to amplify E. coli with a background DNA of Pseudomonas, the result implies that 688 or lower E. coli DAFU/g DS can be detected with the developed protocol. Thus, this method has potential to be used for the quantification of Class A biosolids. Overall, it is concluded that the developed LAMP-DAFU protocol can be used for sensitive and accurate E. coli quantification, and it is especially useful when they are present as a minor population.