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# Rapid Detection of Internal Transcribed Spacer 1 Region of *Opisthorchis viverrini* from Bile Samples in Patients with Cholangiocarcinoma by Loop-Mediated Isothermal Amplification Method

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## Abstract

Opisthorchiasis caused by *Opisthorchis viverrini* is an important fish-borne zoonotic trematode in Southeast Asia. In Thailand, it was found that liver fluke infection is a major cause of Cholangiocarcinoma (CCA). The bile samples were collected from 21 patients with cholangiocarcinoma during June 2014 to February 2015 at the Department of Surgery, Rajavithi Hospital, Bangkok. The internal transcribed spacer 1 of *Opisthorchis viverrini* (OV ITS1-DNA) in bile sample detected by Conventional PCR method showed 5 positive (23.8%) while LAMP and Real-Time PCR methods were 9 positive (42.86%) and 14 positive (66.67%) detected, respectively. In the present study was to detect OV ITS1-DNA by Loop-Mediated Isothermal Amplification (LAMP) methods combined a portable Real-Amp turbidimetric end-point detection method by-product: Magnesium Pyrophosphate ($Mg_2P_2O_7$). The LAMP amplification can be finished in 60 min under isothermal conditions at 63°C in 37 min by employing a set of five species-specific primer mixtures. The results can be checked by naked-eye visualization and 2% agarose gel electrophoresis. The result of lower limit of OV ITS1-DNA detection by naked-eye visualization and 2% agarose gel electrophoresis indicated that the former was able to detect OV ITS1-DNA dilutions up to 2 pg, which it was approximately 10 times more sensitive than 20 pg conventional PCR method. LAMP, Conventional PCR, and Real-time PCR methods were able to detect *O. viverrine*. DNA at a lower level of 1 pg/µL, 10 pg/µL and 100 fg/µL, respectively. The LAMP assay was highly sensitive, and turbidity assay process less than 1 h. A portable turbidimetric system could be easily used at field site. This result may be useful for future research in the detection of *O. viverrini* in bile samples of CCA patients to determine the factors of the causative agent related to CCA with an effective laboratory method.

**Keywords: Loop-mediated isothermal amplification; LAMP; Turbidity; *Opisthorchis viverrini***

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## Introduction

The current incidence of liver fluke infection is a major public health problem in Thailand. Opisthorchiasis caused by *Opisthorchis viverrini* is an important fish-borne zoonotic trematode in Southeast Asia, including Thailand, Lao PDR, Vietnam, and Cambodia [1]. The prevalence of *O. viverrini* was estimated 9 million people are infected with *O. viverrini* in Southeast Asia arises [2]. The association between Cholangiocarcinoma and liver flukes to our knowledge chronic *O. viverrini* infection and the development of CCA [3].

Diagnosis of *O. viverrini* infection by finding the fluke eggs in feces is usually achieved parasitological. Demonstration of eggs in feces, bile, or duodenal fluid or the recovery of flukes during transhepatic stent implantation or from the liver postmortem is considered the "gold standard" for diagnosis of *O. viverrine* [4]. However, parasitological diagnosis alone has many defects from false positive with the eggs of intestinal flukes as morphologically similar to those of *Clonorchis sinensis*

and minute intestinal flukes; it is imperative that requires expertise and particularly false negative in case of light infections and in biliary obstruction in which eggs can't be recovered in the feces [5].

A novel method, termed Loop-Mediated Isothermal Amplification (LAMP) recently developed by Notomi et al. [6] that amplifies DNA with high specificity, efficiency, and rapidity. The LAMP assay is able to amplify a large quantity of target DNA under isothermal conditions [6,7]. This method has been applied successfully for the detection of many pathogenic microorganisms including parasites such as *Plasmodium falciparum* [8], *Acanthamoeba* [9], and *Schistosoma japonicum* [10]. The process reaction of LAMP by product from pyrophosphate ions are released from the Deoxyribonucleotide Triphosphate (dNTP), nucleic acid and magnesium ions in the reaction mix to produce a white, insoluble magnesium pyrophosphate precipitate. This product results in progressively increasing turbidity of the reaction solution. The precipitated product of LAMP can be calibrated the quantitative measurement, the result on the computer screen as a real-time system is presented [11].

A portable Real-Amp Turbidimeter allowed for multi-channel turbidity measurements based on spectroscopic assessment of the magnesium pyrophosphate by product of LAMP reactions [11-13]. No studies have detected OV ITS1-DNA by a potable Real-Amp Turbidimeter. But have also been reported assay for the detection of Shrimp Laem-Singh Virus (LSNV) [11] and shrimp Taura syndrome virus [14]. This is the first report of detection OV ITS1-DNA by LAMP assay using a portable Real-Amp Turbidimeter.

Internal Transcribed Spacers 1 (ITS1) have high level of interspecific sequence divergence which a common mused to detect as molecular marker. ITS1-based tests are particularly useful for the discrimination of the human liver flukes and minute intestinal flukes [15]. Recently, the ITS1-LAMP was specific to *O. viverrini* among the parasites that have been examined with specificity (61.5%) and sensitivity (100%) for rapid diagnosis of *O. viverrini* from stool samples by Arimatsu et al. [12].

The purpose of the present study was examined a sensitivity LAMP detection method combined with a turbidity measurement using an in-house designed portable turbidimeter for diagnosis of *O. viverrini* to evaluate their potential use for a sensitivity LAMP assay.

## Materials and Methods

### DNA Extraction

Total DNA was extracted from adult *O. viverrini* worms using the Pure Gene Core kit A (QIAGEN) and dissolved in a 50 µl elution buffer. The DNA was used for analyzing the sensitivity of the LAMP assay.

### Designing LAMP primers

The primers that target ITS1 were also used to compare the sensitivity [11]. The *O. viverrini* spp. specific LAMP primer set consisted of F3 (Forward outer primer), B3 (Backward outer primer), FIP (Forward Inner Primer), and BIP (Backward Inner Primer).

These primer for the amplification of six distinct regions of the target DNA were set consisting of primers F3, B3, FIP, and BIP was designed using the Primer Explorer V4 (http://www.loopamp.eiken.co.jp) to amplify the *O. viverrini* ITS1 region from Gen Bank accession no EU038151{*Opisthorchis viverrini* isolate OV_A_V2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, complete sequence; and 5.8S ribosomal RNA gene, partial sequence}, 695 bp (https://primerexplorer.jp/elamp4.0.0/Accessed February 21st, 2014). A forward inner primer (FIP, 5-C A G G C C A G C C T G A G A C A C T T T A - C A C C G C C C T G A T G T T G T T G-3′), consisted of F1c (the complementary sequence of F1) and F2, and a backward inner primer (BIP, 5′-T T C A C T G C C C C G A C A T G C A C - T C G T A C C C G G G A T A A G G C-3′) consisted of B1c (the complementary sequence of B1) and B2. The outer primers F3 (5′-T T C G A G C T A C G G C T C A C C-3′) and B3 (5′-C C A A A T G A C C G A G G C G T T A T-3′) and Loop primer F3 (5′-C C G G T G T T C T A C A C T G G A C T-3′). The LAMP primers for *O. viverrini* spp. Specific were designed with similar to Arimatsu et al. [12].

### LAMP Assay

The reaction mixture comprised of Primer Mix (FIP1.6 µM, BIP1.6 µM, F3= 0.2 µM, B3 0.2 µM, and LB 0.8 µM), Bst DNA polymerase (8 U; New England BioLabs), 20 mM Tris–HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 8 mM $MgSO_4$, 0.1% Triton X-100, 1.6 M Betaine, 1.4 mM deoxynucleotide triphosphates each, and template DNA (2 µL). Double-distilled water was finally used to fill the system to 25 µL. No template DNA was added in the negative control reaction with 15 µl of mineral oil (Sigma-Aldrich, Steinheim, Germany) to prevent evaporation of components. The mixture was incubated at temperature optimization was carried out at 60, 63 and 65°C using a portable Real-Amp for shrimp diagnosis Turbidimeter (Mobilis Automata, Thailand) for 60 min.

### Conventional PCR with outer primers F3 and B3

Conventional PCR was performed with the outer primers F3 and B3 which were used for amplification of *O. viverrini* ITS1 region in the LAMP assay. The PCR reaction was carried out in a 25 µL system with 10× PCR buffer (2.5 µL), 0.2 mM of each dNTPs, 0.4 µM of each B3 and F3 primers, and 1.25 U of i-Taq DNA polymerase (Intron biotechnology), and 2 µL of DNA sample in a thermocycler (BIOER Little genious) under the following conditions: After an initial denaturation at 95°C for 7 min, then 35 cycles of denaturation (30 s at 95°C), annealing (30 s at 60°C), and extension (30 s at 72°C), and a final extension for 7 min at 72°C. Five microliters of PCR products (213 bp) was examined on a 2% agarose gel electrophoresis.

### Real-Time PCR with outer primers F3 and B3

Conventional PCR was performed with the outer primers F3 and B3 which were used for amplification of *O. viverrini* ITS1 region in the Real-time PCR assay. The homology search of the *O. viverrini* sequence was performed by BLAST search program at the National Center of Biotechnology Information (NCBI), U.S.A. Genbank accession number of EU038151{*Opisthorchis viverrini* isolate OV_A_V2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, complete sequence; and 5.8S ribosomal RNA gene, partial sequence}, 695 bp. The Real-time PCR reaction was carried out in a 20 µL containing SYBR Green Fast Universal qPCR Kit (KAPA Bio systems), 0.2 µM each primer and 2 µL of DNA sample in a Chromo 4™ System (Bio-Rad, U.S.A) was performed (MJ Opticon Monitor ver. 3.1). Master Mix for Real-time PCR was prepared with 10× PCR buffer (2.5 µL), 0.2 mM of each dNTPs, 0.2 µM of each B3 and F3 primers (0.4 µl each), and 10 µl of i-Taq DNA polymerase (Intron biotechnology), and 2 µL of DNA sample in a thermocycler (BIOER Little genious) under the following conditions: after an initial denaturation at 95°C for 7 min, then 35 cycles of denaturation (30 s at 95°C), annealing (30 s at 60°C), and extension (30 s at 72°C), and a final extension for 7 min at 72°C. Melting Curve from 65°C to 95°C,

hold 5 sec was determined [13].

After amplification, the Real-Time PCR products were melted by raising the temperature from 65°C to 95°C, with an increment of 0.5°C/sec, in order to obtain information on melting profiles. Melting Temperature (Tm) was determined by melting curve analysis. Distilled water was used as a negative control for these assays. The Tm values were 85°C to 86°C.

### Limit detection of LAMP and PCR

To compare test sensitivity, 10 fold diluted of the total DNA (14 ng to 200 fg) was used as template for LAMP using pre-determined conditions. After the reaction, LAMP product was detected by visualized, portable Real-Amp turbidimeter, and 2% agarose gel electrophoresis.

## Results

### LAMP conditions

Test LAMP by portable Real-Amp turbidimeter at 60°C, 63°C, 65°C for 60 min using 14 ng of total DNA as template revealed that LAMP products could be visualized at all tested temperatures. For reaction time, the turbidity was seen first at 45 min and then at 37 min and 40 min at 65°C, 63°C and 60°C, respectively. Therefore, temperature and reaction time at 63°C for 60 min were selected as the optimal conditions for the LAMP assay.

### Limit detection of LAMP and PCR

Test the sensitivity of the LAMP assay. The concentration of a total DNA of *O. viverrini* was measured by spectrophotometry two times to obtain an average concentration. A final concentration of 7 ng/µl. Ten-fold serial dilution were prepared ranging from $1 \times 10^{2}$ to $1 \times 10^{-4}$ ng/µl and used as template for amplification by LAMP using a portable Real-Amp turbidimeter and conventional PCR. After the amplifications, the products were visually inspected and/or detected on a 2% agarose gel and the sensitivity of the two assays was compared.

The LAMP assay with a portable Real-Amp turbidimeter was able to detect OV ITS1-DNA at a 2 pg. This corresponded with the sensitivity of LAMP followed by gel electrophoresis [14].

When the LAMP assay with the turbidimeter was used DNA

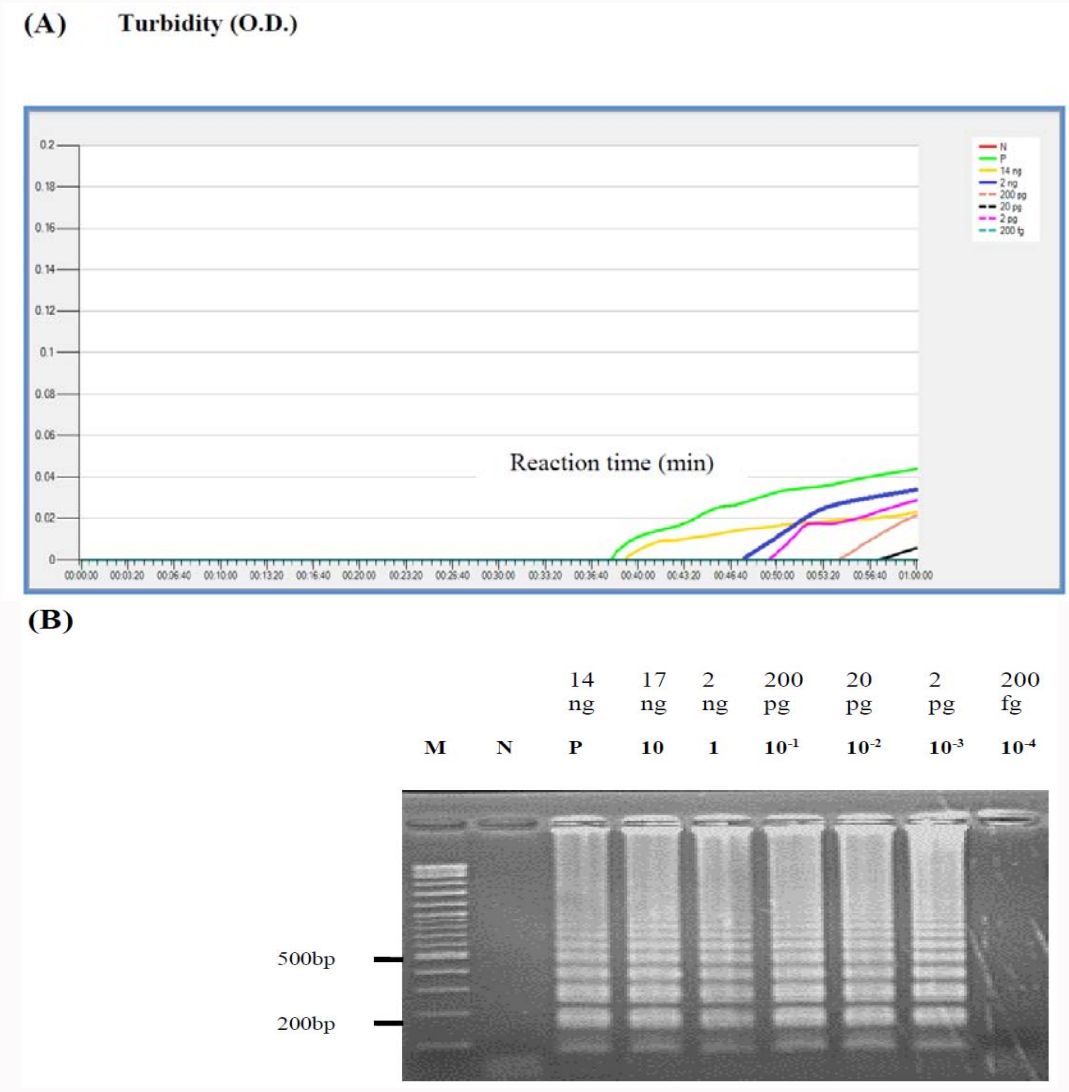


**Figure 1:** Comparative sensitivity of OV ITS1-DNA by LAMP using a portable Real-Amp turbidimeter was conducted at 63°C for 1 hr. Then followed by gel electrophoresis. (A) Turbidimeter plots from assays using 10 fold serial dilutions of DNA extracted from *O. viverrini.* (B) Gel electrophoresis from the same serially diluted DNA. Lane M: 2 log DNA marker Lane N: No template DNA and Lane P: Total DNA.

**Table 1:** Comparison the sensitivity of four methods to the low limit of detection of *O. viverrini*.

| | $10^2$ (14 ng) | 10 (7 ng) | 1 (2 ng) | $10^{-1}$ (200 pg) | $10^{-2}$ (20 pg) | $10^{-3}$ (2 pg) | $10^{-4}$ (200 fg) |
|---|---|---|---|---|---|---|---|
| LAMP-visualized | + | + | + | + | + | + | - |
| LAMP-Gel electrophoresis | + | + | + | + | + | + | - |
| LAMP-turbidimeter | + | + | + | + | + | + | - |
| Conventional PCR | + | + | + | + | + | - | - |

*(−): Not amplified; (+): Amplified

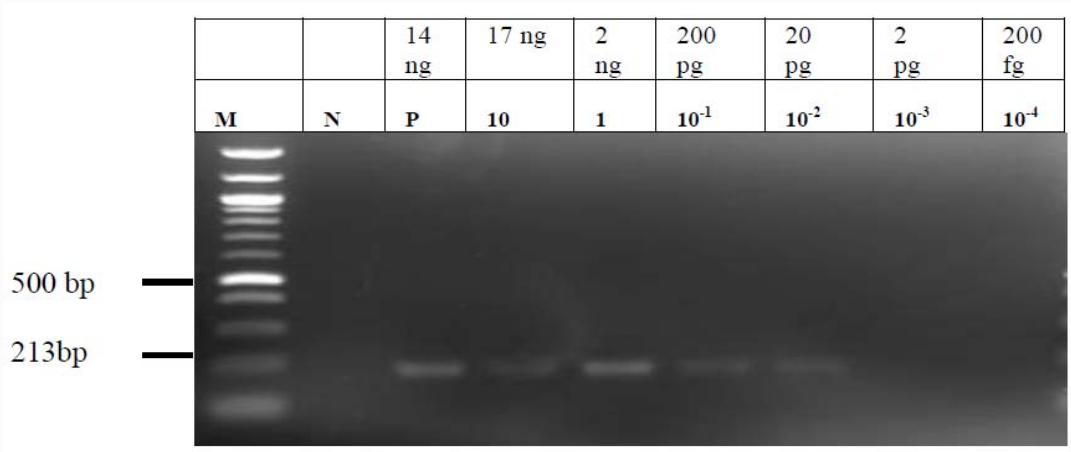


**Figure 2:** Sensitivity test of conventional PCR using LAMP outer primers (F3, B3) was detect OV ITS1-DNA template 20 pg. Lane M: 2 log DNA marker Lane N: No template DNA and Lane P: Total DNA.

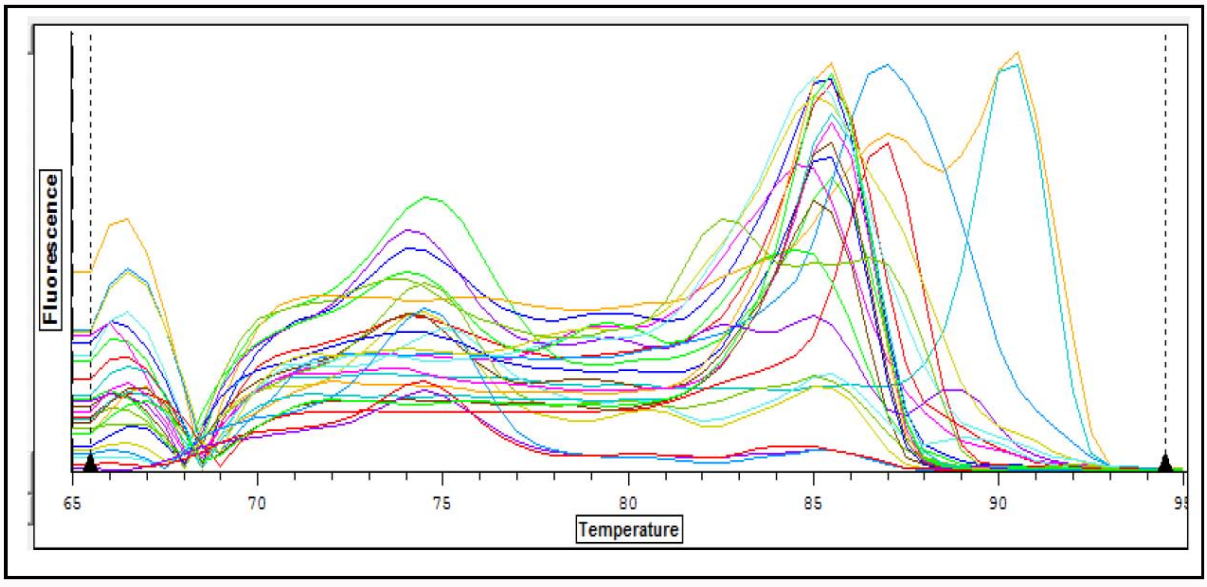


**Figure 3:** The melting curve analysis of Real-time PCR end products of the *Opisthorchis viverrini* ITS1 DNA which were obtained in bile sample. The melting temperature of *O. viverini* was approximately 85.5°C.

template concentrations it was able to detect lower limitation at 2 pg of total DNA (Figure 1A). This corresponded with the sensitivity of LAMP followed by 2% agarose gel electrophoresis (Figure 1B) and visualized (Table 1), and both were 10-times more sensitive than conventional PCR detection. The PCR method was lower limit detected at 20 pg of OV ITS1-DNA (Figure 2).

The amount of *Opisthorchis viverrini* ITS1 DNA in bile samples of patients with Cholangiocarcinoma (CCA) detected by Real-time PCR.

The amount of *O. viverrini* ITS1 DNA in bile sample was determined by real-time PCR using SYBR Green. The melting curve analysis showed that *O. viverrini* melting temperature was approximately 85.5°C (Table 2) [15]. CCA group, *O. viverrini* DNA were presented in 14 of 21 cases (66.67%). In CBD group, cholangitis group and HCC group, bile sample were detected by using the same method as CCA group. All bile samples were negative from *O. viverrini* (Figure 3).

**Table 2:** The melting temperature of *Opithorchis viverini* ITS1 DNA in bile samples from 21 patients with Cholangiocarcinoma (CCA).

| Patients Group | Case No. | Real-Time PCR Using SYBR Green 1 | |
|---|---|---|---|
| | | ***Tm* 85-86°C** | **Result** |
| Control | Negative | 74.5 | Negative |
| | Positive | 85.5 | Positive |
| CCA (n=21) | 1 | 86 | Positive |
| | 2 | 86 | Positive |
| | 3 | 85.5 | Positive |
| | 4 | 91 | Negative |
| | 5 | 85 | Positive |
| | 6 | 90.5 | Negative |
| | 7 | 74.5 | Negative |
| | 8 | 85.5 | Positive |
| | 9 | 75.5 | Negative |
| | 10 | 85.5 | Positive |
| | 11 | 74.5 | Negative |
| | 12 | 87 | Negative |
| | 13 | 87 | Negative |
| | 14 | 85.5 | Positive |
| | 15 | 85.5 | Positive |
| | 16 | 85.5 | Positive |
| | 17 | 86 | Positive |
| | 18 | 85.5 | Positive |
| | 19 | 85 | Positive |
| | 20 | 85.5 | Positive |
| | 21 | 85 | Positive |
| CBD | 1 | 82.5 | Negative |
| CT | 1 | 75 | Negative |
| HCC | 1 | 74.5 | Negative |

## Discussion

The amount of *Opisthorchis viverrini* ITS1 DNA in bile samples from patients with Cholangiocarcinoma (CCA) analyzed by Loop-mediated isothermal amplification of DNA (LAMP).

The hypothesis that this study found *O. viverrini* DNA in bile samples in patients with Cholangiocarcinoma may be adult parasite lives in the biliary ducts, sucking and release certain of substances out, cause irritation to the biliary tract then inflammatory repeatedly inserting the key cause is CCA [15]. This result showed that *O. viverrini* DNA in bile from patients with CCA. A previous study, Qiao et al. [16] have developed of Real-Time PCR assay for the detection of *C. sinensis* DNA in gall bladder bile and stone samples from patients with cholecystolithiasis, the results showed that eggs-positive bile samples determined by microscopy yielded positive results by Real-time PCR assay. There for, we inferred that the bile is an important site for adult ovulation because the egg in the bile are typically discharged to intestinal tract a periodically [16]. The human liver flukes, *O. viverrini*, *O. felineus* and *C. sinensis* remain important public health problems in many parts of the world [17], the result in severe morbidity of biliary and hepatic diseases, particularly CCA-the bile duct cancer [11]. Interestingly, this is the first report of detection of *O. viverrini* using a LAMP-turbidimeter. Previous report of LAMP-Turbidimeter for Shrimp Viral Detection, the equipment is available under the trade name "Real-AMP". Arunrut et al. [11] have reported that the first report of quantitative detection of Shrimp Laem-Singh Virus (LSNV) using a real-time RT-LAMP assay and Sappat et al. [14] have development of RT-LAMP combined end-point turbidity measurement using a designed apparatus for detection of shrimp Taura Syndrome Virus (TSV). Moreover, the LAMP-turbidimeter is suitable not only for the detection of LSNV and TSV but also for many pathogen of shrimp pathogen of shrimp such as White Spot Syndrome (WSSV), Acute Hepatopancreatic Necrosis Syndrome (AHPNS). Turbidimeter works by shooting a red-light bulb LED. Light is shot through the solution hits a sensor. The amount of incident light will depend on turbidity of the solution. If the solution is cloudy, the DNA samples were examined for the disease read result on PC, notebook, netbook. The result can be achieved through the real-time semi-quantitative method. Positive result at the lowest detection level and the negative is very clear in turbidimeter. The real-time turbidity method is qualitative monitoring with automate, not requiring probe/indicator or other reagents. The risk of amplicon contamination is eliminated. We suggest that turbidimeter is a rapid method and most practical due to its ease of application in a basic laboratory.

The amount of *Opisthorchis viverrini* ITS1 DNA in bile samples from patients with Cholangiocarcinoma determined by conventional PCR, Loop-mediated isothermal amplification and real-time PCR methods.

In this study, *O. viverrini* DNA in bile samples was detected by conventional PCR method, the result showed 5 positive (23.8%) and 16 negative (76.2%) out of 21 samples. Arimatsu et al. [18] have reported that the ITS1-PCR showed 9 positive (18%) and 41 negative (82%) out of 50 samples, it was specific to *O. viverrini* with sensitivity of 24.3% and specificity of 100% using the microscopic examination as a gold standard. LAMP and conventional PCR were compared by using the same DNA, the positive of *O. viverrini* of LAMP assay were consistently higher than these of conventional PCR in the bile sample. Arimatsu et al. [18] have reported that the ITS1-LAMP showed 42 positive (84%) and 8 negative (16%) out of 50 samples, it was specific to *O. viverrini* with a sensitivity and specificity of 100% and 61.5%, respectively using the microscopic examination as gold standard. Le et al. [19] respected that the specific LAMP assay was used to detect eggs from feces. The mito-OvLAMP is 100 times higher sensitive than the PCR assay [19]. In this study, a portable turbidimetric end-point detection method was established for the detection of *O. viverrini* using LAMP, *O. viverrini* ITS1 DNA can be found in 9 of 21 bile samples from CCA patients. The relative merits of LAMP and PCR methods for the detection of *O. viverrini* were discount. LAMP method is a simple and rapid molecular biology diagnostic tool with high sensitivity. LAMP method was designed by primer V4.0 is rapid DNA amplification to sensitivity. The specificity of the LAMP amplification is directly attributed set of five specially designed primers that recognize a total or six distinct sequences on the target DNA whereas conventional PCR has two primers. LAMP method has reaction tube that contains buffer as well as target DNA, Bst DNA polymerase, and primers. A portable turbidimeter was a rapid method, which can obtain result with 1.15 h to maintain the temperature at 63°C. There is no need for a thermal cycler. Moreover, clear difference between the negative control and positive sample was detained. The final product amplified by the LAMP assay is detected by unaided visual examination. The time required for amplification

detection of the product is about 60 min and no need for gel electrophoresis whereas that for PCR is 3 h to 4 h.

LAMP and Real-time PCR analysis of *O. viverrini* ITS1 DNA in bile samples were compared Real-time PCR detected 14 positive (66.67%) and 7 negative samples (33.33%) out of 21 samples. Meanwhile, LAMP detected 9 positive (42.86%) and 12 negative samples (57.14%) out of 21 samples. Over all, the percentage of *O. viverrini* positive by Real-time PCR was higher than LAMP methods. Real-time PCR has more advantage than the other diagnosis molecular methods. According to previous studies, Boonma et al. [20] have studied multiplex PCR, nested PCR and Real-time PCR assay for the detection of *P. falciparum* and *P. vivax* from clinical blood spots dried on filter paper; they have found that the real-time PCR methodology was the most sensitive method. Reddy et al. [21] found a good correlation between the results of the real-time PCR and LAMP assays for the detection of CMV. The Real-time PCR with specific primer had been developed for the detection of *O. viverrine* [22]. Real-time PCR can be used widely in diagnostic method, but it has some limitations such as expensive substrates and need thermal cyclers machine [20,21].

### Comparison of low limited detection in Conventional PCR, LAMP and Real-time PCR methods

Since the outer primer pair, designated F3 and B3 can also be used for Conventional PCR and Real-time PCR, the same target gene was amplified from serially diluted *O. viverrini* DNA. The low limited detection of the three methods was compared.

The limit of detection of the LAMP-turbidimeter was 1 pg/μL consistent with LAMP assay using SYBR green I of 3 pg/μL [19] and LAMP using hydroxy naphthol blue was 1 pg/μL [23]. Regarding the sensitivities of the three different LAMP methods, similar results were obtained because targeting ITS 1 gene of *O. viverrini*. Targeting ITS1 was successful to perform a specific amplification, whereas Le et al. [19] have report the mitochondrial nad1 gene using LAMP-SYBR green I as little as 100 fg/μL of DNA template [20], it is possible that the targeting mitochondrial nad1 gene of *O. viverrini* was more appropriate ITS1 to using LAMP.

Conventional PCR was 10 pg/μL. The low limited detection of the conventional PCR was 10 times lower than LAMP-turbidimeter [24]. According to previous studies Chen et al. [25] have studied the sensitivity of the LAMP assay for the detection of *P. westermani* DNA was 100 time higher than that of conventional PCR [24]. Different from the result of Real-time LAMP assay using SYBR green I similar sensitivity was observed by Conventional PCR [19].

The detection limit of the Real-time PCR with SYBR green I using F3 and B3 primers was 100 fg/μL. A previous study, Real-time PCR method targeting the COX1 gene showed good sensitivity, but it was 1 pg/μL for *O. viverrini* of genomic DNA [25]. In this study, Real-time PCR method targeting the ITS1 gene of *O. viverrini* was successfully developed. Real-time PCR using the outer primers (F3 and B3) were performed on each DNA template of *O. viverrini* and anneal to the template strand and also generate new DNA.

These results also suggest that, LAMP can be used for the detection of *O. viverrini.* Moreover, LAMP requires only a turbidimeter that providing a constant 63°C for 1 h during which turbidity was continuously measured and quantitative results could be obtained by reaction time measurement [11], which makes it more economical and practical than PCR. The white turbidity of magnesium pyrophosphate accumulation as by-product of DNA amplification can be detected by naked eye or by turbidimeter.

The duration of work itself, is of the opinion that those who have conducted research using conventional, LAMP and Real-time PCR techniques will require skilful and cautious. Real-time PCR require thermal cycles and skill technicians. The LAMP and PCR technique is contaminating the reaction is simple so need to be attentive very carefully during the trial and to be aware of the contamination of certain chemicals used in the trial. Most of which are carcinogens such as Ethidium bromide, etc., must be attentive to the tools used in conjunction with others to avoid such contamination. Cleaning and maintenance tools in good working condition at all times.

## Conclusion

In conclusion, the new LAMP method has is highly sensitive of OV ITS1-DNA. LAMP is emerging as a promising new method due largely to its low cost, ease of operation, and high sensitivity. The advantage as a diagnostic test which can assist in bringing point-of-care diagnosis to patients. Future studies will be aimed to apply of this LAMP procedure for routine diagnosis of opisthorchiasis.

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