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Enhancing the forensic sexual assault investigations with LAMP-based male DNA detection

Sunita Chunkul [a], Tikumphorn Sathirapatya [b,d], Piyawan Dangklao [c], Praphat Kawicha [e], Rachaneekorn Tammachote [a,*], Kornkiat Vongpaisarnsin [b,c,d]

[a] Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok, Thailand
[b] Department of Forensic Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
[c] Forensic Serology and DNA, King Chulalongkorn Memorial Hospital and Thai Red Cross Society, Bangkok, Thailand
[d] Center of Excellence in Forensic Genetics, Ratchadapiseksompotch Fund, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
[e] Plant Genome and Disease Research Unit, Department of Agriculture and Resources, Faculty of Natural Resources and Agro-Industry, Kasetsart University, Chalermphrakiat Sakon Nakhon Province Campus, Sakon Nakhon, Thailand

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ABSTRACT

Sexual assault is a terrible crime that demands a comprehensive and skilled investigation to gather the perpetrator's biological material. To collect all possible physical and biological evidence, forensic investigation is crucial and should be conducted as soon as possible. The primary focus of such investigations is the detection of male-specific materials. This study presents a novel assay utilizing the LAMP technique to detect male DNA. The assay underwent validation following the SWGDAM guidelines and was subsequently tested on 92 casework samples from sexual assault cases. To evaluate its performance, the outcomes of three distinct tests: acid phosphatase, microscopic examination, and the LAMP assay, were compared against the Y-STR profiling results. The LAMP assay exhibited remarkable efficiency, comparable to Y-STR profiling. These findings emphasize the LAMP technique's potential as a valuable tool for male DNA detection in forensic casework. Further research and validation studies are essential to fully explore its practical applications and enhance its utility in criminal investigations.

1. Introduction

Sexual assault is one of the most serious crimes that requires immediate attention in forensic investigations. This type of crime often causes severe physical and psychological trauma to victims. Identifying even the smallest trace in each investigation is crucial. Verification of the presence of male trace in sexual assault evidence can be a critical decision point in determining whether to proceed with DNA analysis. Commonly employed presumptive tests involve seminal acid phosphatase (AP) or prostatic-specific antigen (PSA) tests [1]. Although AP shows high sensitivity in identifying seminal fluid, it can produce false positive results due to its low amount in vaginal secretions and other biological fluids [2]. To increase the accuracy of semen detection, alternate tests such as semenogenin and zinc tests have been proposed [1,3]. The technical transition from chemical testing to immunochrographic-based method, such as ABAcard® p30 for PSA, has also shown promising results [4]. It is important to note that AP and PSA do not directly indicate the presence of male cells; instead, a positive result reveals the presence of an enzyme or protein in the seminal fluid, which is not a promise of DNA recovery for identification purposes.

An alternative approach involves detecting a male cell and obtaining DNA for identification. Several DNA-based methods have been developed, including real-time PCR [5–7] and differential extraction. The latter method is used to differentiate between spermatozoa cells and female cells, offering high specificity regardless of the presence of spermatozoa [8]. Microscopic examination of spermatozoa is highly specific and considered the "gold standard" or confirmatory test, but it can be time-consuming and require interpretation by an experienced scientist, especially in cases of azoospermia [1,3].

Loop-mediated isothermal amplification (LAMP) has been used as a point-of-care diagnostic tool to detect pathogens, including SARS-CoV-2 [9,10]. LAMP provides several advantages for forensic investigation.

* Corresponding author. Department of Botany, Faculty of Science, Chulalongkorn University, 254 Phaya Thai Rd, Wang Mai, Pathum Wan, Bangkok, 10330, Thailand.
E-mail address: ratchaneekorn.t@chula.ac.th (R. Tammachote).

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The applications in forensic fields include male DNA identification [11–13], human DNA identification [14], body fluid identification [15], *Canabis sativa* [16] and poisonous mushroom [17]. The reaction relies on four to six primers to amplify the target sequence exponentially, providing high specificity and sensitivity [9,10,18]. These primers are designed to anneal at different nucleotide position encompassing the target sequence. With the use of DNA polymerase strand displacement activity, at single incubation temperature, the whole process occurs including denaturing, annealing and elongation simultaneously (Supplement Fig. 1). Furthermore, LAMP requires minimal equipment compared to conventional PCR, making it suitable for field-based detection with simple colorimetric indicators such as hydroxy naphthol blue (HNB) or phenol red that can be easily observed with the naked eye. In addition, real-time LAMP assays are performed by monitoring the fluorescence signal [19]. Therefore, the objective of this study is to explore the potential of the LAMP technique in the detection of male trace DNA in the sexual assault casework. The LAMP assay was optimized and validated according to the SWGDAM validation guidelines. The results suggest that the LAMP assay is a promising tool for detecting male trace DNA in sexual assault casework.

2. Materials and methods

Ethical approval

All procedures were performed in accordance with the guidelines of the Helsinki Declaration. This study did not involve the collection of identifiable private information and is exempt from the need for ethical approval under 45CFR 46.101(b) by the Institutional Review Board (IRB) of the Faculty of Medicine, Chulalongkorn University (IRB No.115/64).

2.1. Primer design

LAMP primers were designed to anneal specifically to human AMELY (accession number NG_008011.2). The FASTA file of human AMELY was used as a template sequence in the LAMP Designer 1.16 program (http://www.optigene.co.uk/lampdesigner/) using the following parameters: length in 18–22 bp, 65 ± 2 °C for Tm of the F1c and B1c primers, 62 ± 2 °C for Tm of the LF and BF primers, distance between 5′ ends of F2 and B2 in the range of 120–180 nucleotides, distance between 5′ ends of F2/B2 and F1c/B1c in the range of 40–60 nucleotides, distance between 3′ ends of F3/B3 and 5’ of F2/B2 in the range of 0–60 nucleotides, and % GC in the range of 40–65. Six LAMP primers used in this study are described in Table 1 and their nucleotide positions are shown in Supplement Fig. 2.

2.2. Samples

FLOQSwabs® with 100 mm of break point (Copan) was used to collect buccal cells from male (N = 5) and female (N = 5) volunteers. All samples were then subjected to DNA isolation using the Prepfiler™ Forensic DNA extraction kit (Applied Biosystems). Isolated DNA was quantified by Quantifluor® ONE dsDNA system (Promega) and stored at −20 °C until use. These samples were for LAMP optimization. The optimal LAMP condition was then used in all subsequent tests.

For the sensitivity test, serial dilutions (10-fold) of male DNA (1, 0.1,0.01 and 0.001 ng) were carried out in triplicate LAMP reaction to define the lowest DNA input that can give a positive result. In addition, one azoospermia semen sample was used for a challenging sample that may contain a small amount of male DNA. Male specificity was tested using female DNA as a negative control along with nuclease-free water as a blank control.

For the species specificity test, house mouse (*Mus musculus*), brown rat (*Rattus norvegicus*), and pig (*Sus scrofa domestica*) DNA samples were taken as domestic mammals. Five primate species (n = 22) were examined for cross reactivity in LAMP assay.

For the blind test, the first scientist prepared ten mixture samples and then the second scientist carried out from DNA isolation through data interpretation. These mixture samples were prepared by random combination of male blood, female blood, male saliva, female saliva and male semen. The key combinations and their amounts were noted and compared to the LAMP results. DNA extraction was carried out using the Prepfiler™ Forensic DNA extraction kit (Applied Biosystems). The isolated DNA was quantified using the Quantifluor® ONE dsDNA system (Promega).

For stability test, a single drop of semen was deposited on three fabric types in approximate one square inch to create mocked biological specimen that obtained from sexual assault case. Genomic DNA was extracted by using Prepfiler™ Forensic DNA extraction kit (Applied Biosystems) and was quantified using the Quantifluor® ONE dsDNA system (Promega).

2.3. Casework samples

To validate the compatibility with the casework samples, the remaining vaginal swab samples (N = 92) from sexual assault cases subjected to AP and ME at the Forensic Serology and DNA unit, King Chulalongkorn Memorial Hospital and the Thai Red Cross Society were used as casework samples. DNA extraction was carried out in all casework samples using the Prepfiler™ Forensic DNA extraction kit (Applied Biosystems). Isolated DNA was quantified using the Quantifluor® ONE dsDNA system (Promega) and stored at −20 °C until use.

2.4. LAMP reaction

The LAMP reaction composed of 12.5 μl of 2x WarmStart Colorimetric LAMP Master Mix with UDG (New England Biolabs), 2.5 μl of LAMP primer mix (containing 1.6 μM of FIP and BIP primers, 2 μM F3 and B3 primers, and 4 μM Loop F and Loop B primers), and 1 μl of DNA template in a total reaction volume of 25 μl. Amplification was performed at 65 °C using the Proflex PCR system (Applied Biosystems).

The LAMP reaction results were visualized using two methods. The first method involved observing the color of the LAMP reaction after incubation. A pink color indicated a negative LAMP reaction, while a transition from pink to yellow indicated a positive LAMP reaction. Subsequently, the reaction was confirmed using the QIAxcel DNA High Resolution Kit (QIAGEN) with the QIAxcel Advanced system (QIAGEN). The QX Alignment Marker 15bp/3 kb was employed along with the QX DNA size marker 50–800 bp v2.0 (QIAGEN).

Table 1
Sequence and length of six LAMP primers [20].

Primer	Sequence 5′ to 3′ direction	Length (bp)
F3	CAGTGGCTTAAGGTATGGTTAT	22
B3	GGACCTGAGAATCCACCT	18
FIP	TGATGGCTATGAACAGGGATGCGATACCAGGTAGGTTAATGCC	43
BIP	ACAGGCACCAATGTTACTGGGGCTCTCACTACCAGTGTCTA	41
LF	TTGATGTACCACTGCTACTGG	21
LB	ATTCTTGGACCACTGGACG	19

2.5. Acid phosphatase and microscopic examination

The vaginal swab of each case was analyzed using two methods: an acid phosphatase (AP) test and microscopic examination (ME). For the AP test, the swab sample was moistened with distilled water and pressed onto filter paper. A few drops of α-nitrophenyl phosphate (Sigma-Aldrich) were applied to the filter paper. A positive result was indicated by the development of a purple color within 60 s. For ME, the glass slide format was used. The sample was subjected to hematoxylin and eosin (H&E) staining prior to microscopic examination. A positive result was observed when at least one intact spermatozoon was present under the microscope.

2.6. Y-STR profiling

One nanogram of isolated DNA sample from sexual assault casework was amplified by either Yfiler™ Plus PCR Amplification Kit (Applied Biosystems) or PowerPlex® Y23 System (Promega). The thermal cycling conditions were followed according to the manufacturer's instructions for each kit. The Y-STR amplicon was injected for 24 s at 1.5 kV on ABI® 3500 Genetic Analyzer (Applied Biosystems). Allele designation was analyzed by GeneMapper™ ID-X 1.6 software (Applied Biosystems) with analytical threshold of 150 relative fluorescence units.

2.7. Statistics

The efficiency of three different methods for male-specific detection that were AP, ME and LAMP tests was compared. Sensitivity, specificity, positive predictive value (PPV), negative predictive (NPV) and accuracy of each method were evaluated based on Y-STR profiling results as a gold standard for male-specific detection. The receiver operating characteristic curve (ROC) and area under the curve (AUC) were evaluated for performance of three test. The correlation of the AP test, ME and LAMP results with the Y-STR profile results was assessed by chi-square test in the 2x2 Table. The accuracy was determined by the sum of true positive and true negative dividing by the sum of all results. Precision or positive predictive value (PPV) was calculated by true positive dividing by the sum of true positive and false positive.

2.8. Case study

A case of sexual assault subjected for AP and ME at the Forensic Serology and DNA, King Chulalongkorn Memorial Hospital and Thai Red Cross Society. Eight swabbing samples were collected from different areas and all samples were subjected to the LAMP test and Y-STR profiling.

3. Results

3.1. LAMP optimization

The optimization of the LAMP reaction involved a comprehensive evaluation of various factors, including the type of LAMP master mix, amplification temperature, incubation time, and concordance between two detection methods, phenol red colorimetric detection and the QIAxcel Advanced system (QIAGEN). Based on the evaluation, the WarmStart® Colorimetric LAMP master mix with UDG (NEB) was chosen, and the manufacturer's instructions for the LAMP reaction were strictly followed. Two observation methods were used at 30 and 45 min. The first method utilized the colorimetric phenol red present in the LAMP master mix. A pink color indicated a negative LAMP reaction, while a color transformation from pink to yellow indicated a positive LAMP reaction. The second method involved the QIAxcel Advanced system (QIAGEN) with the QIAxcel DNA High Resolution Kit (QIAGEN). A positive result displayed a ladder-like pattern, whereas a negative result showed a blank in the electropherogram. The consistency between these two detection methods was closely monitored. According to the results, the WarmStart® Colorimetric LAMP master mix (NEB) with incubation at 65 °C for 30 min demonstrated remarkable consistency and reproducibility in analyzing five male and five female buccal swab samples (Fig. 1). This consistency was also observed at the 45-min time point. All male samples exhibited positive LAMP results, while all female samples showed negative results. In particular, the phenol red colorimetric detection showed results consistent with the electrophoresis-based detection using the QIAxcel (Fig. 1). Therefore, the optimized LAMP reaction was applied for all subsequent tests.

3.2. Sensitivity and specificity tests

To determine the lowest detectable DNA input, a series of 10-fold serial dilutions of male DNA (1, 0.1, 0.01, and 0.001 ng) were subjected to triplicate LAMP reactions. The reaction was carried out separately for 30- and 45-min incubation time using the visible phenol red and QIAxcel Advanced system (QIAGEN). Negative results were observed within triplicates when the DNA template was less than 0.1 ng (Fig. 2). However, inconsistent results were observed in triplicates of 0.01 and 0.001 ng of male DNA templates. One of the replicates exhibited an orange transition color of phenol red at 30-min (Fig. 2), which turned yellowish after an additional 15 min of incubation (data not shown). Therefore, the sensitivity of the test was determined to be 0.1 ng of male DNA template. An azoospermia sample was examined by AP test, microscopic examination and LAMP assay. Both AP and microscopic examination had positive results. The isolated DNA of azoospermia specimen was small (0.04 ng/μl). Hence, 2 μL of isolated DNA were used and LAMP assay still gave negative result (data not shown).

In terms of species specificity, the LAMP test showed negative results for house mouse (*Mus musculus*), brown rat (*Rattus norvegicus*) and pig (*Sus scrofa domestica*) DNA samples (Supplement Fig. 4). For primates, in the group of Macaca genus, positive results were scattered among male and female samples. Two male samples of *Pongo pygmaeu* also showed negative results, while the positive result was observed in male *Pan troglodytes* and demonstrated negative result in female sample (Supplement Table 1).

3.3. Blind test

To evaluate the effectiveness of the LAMP test in identifying male DNA within complex mixtures, another scientist prepared ten mock samples consisting of a mixture of semen, male saliva, male blood, female saliva, and female blood(Table 2). Subsequently, DNA extraction, LAMP amplification and interpretation were carried out by another scientist. The LAMP results were consistent across both detection methods. Mixture numbers 3 and 9 showed negative for male DNA since these mixtures did not contain any male biological fluids (Table 2). The LAMP reaction accurately identified all male DNA mixtures, regardless of the type of biological fluid present.

3.4. Stability test

Three different texture and color of fabrics were deposited with one drop of semen to imitate specimens obtaining from sexual assault cases. After the DNA isolation, one of the samples (fabric no.3) showed turbid and yellowish elutant while other two samples showed clear and colorless elutant. All sample showed positive results for LAMP assay (Supplement Fig. 4).

3.5. Casework samples

To validate the compatibility of the LAMP test with forensic samples, the remaining vaginal swab samples (N = 92) from sexual assault cases were subjected to the LAMP reaction and Y-STR profiling. These samples

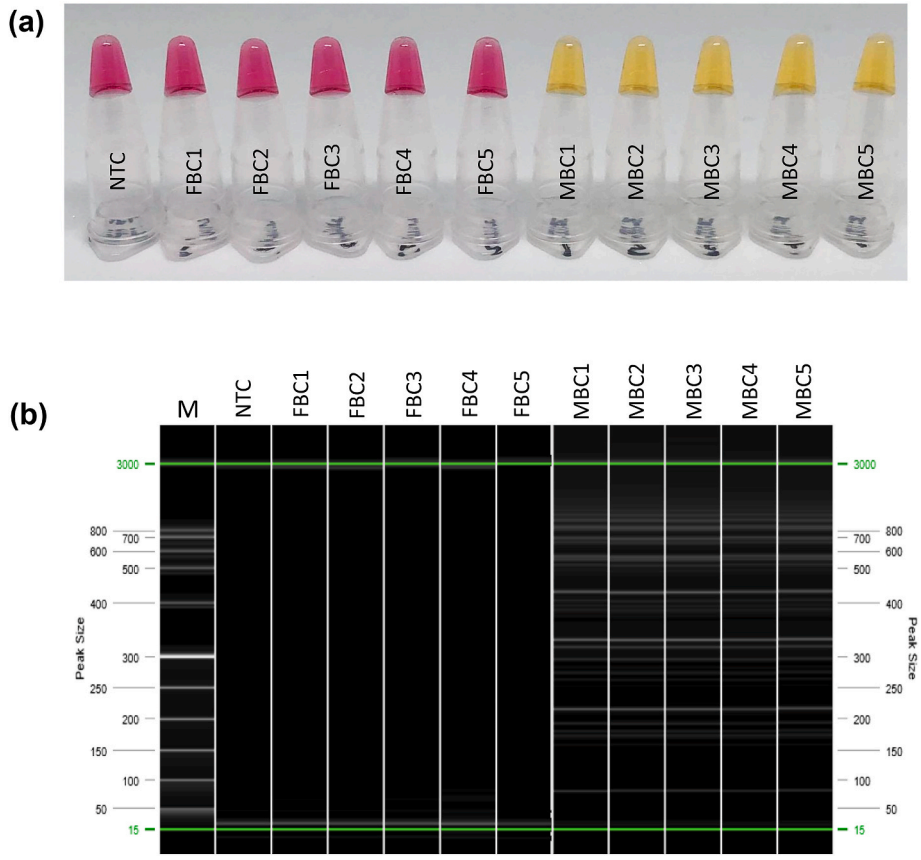


Fig. 1. LAMP detection of no template control (NTC), female (FBC1-FBC5) and male (MBC1-MBC5) buccal swab samples (N = 10) by Phenol red (a) and QIAxcel (b) Advanced system (QIAGEN). LAMP reactions were carried out at 65 °C for 30 min. M indicates QX DNA size marker 50–800 bp v2.0 (QIAGEN).

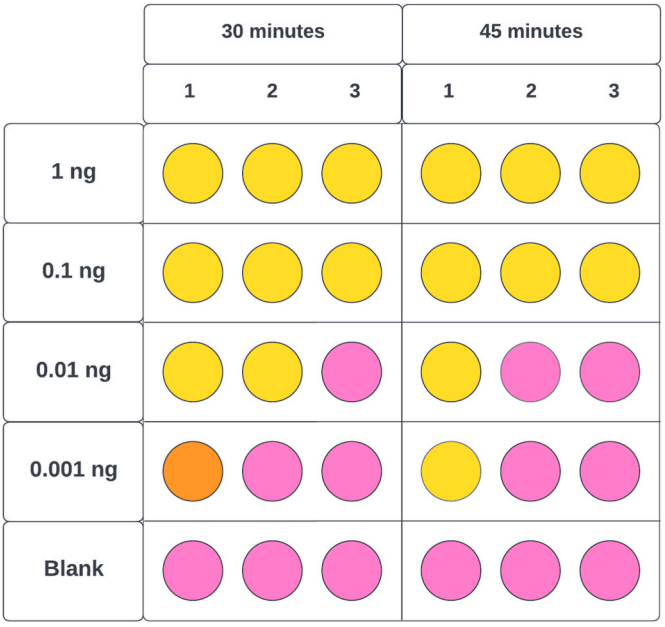


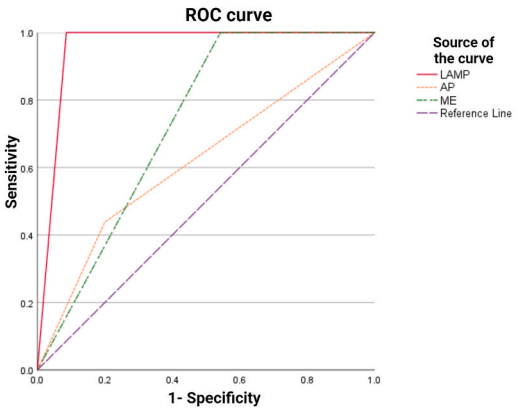
Fig. 2. The sensitivity of the LAMP reaction was tested using four serial dilutions of male DNA samples ranging from 1 ng to 0.001 ng. The LAMP reactions were performed at 65 °C for 30 and 45 min in triplicate. The amplicons were observed using visible phenol red and QIAxcel Advanced system (QIAGEN). The color in circular objects indicated the results of phenol red.

Table 2
A blind test of the LAMP reaction was performed using ten mock samples prepared by another scientist. The amount and composition of each mock sample was recorded. The LAMP reactions were performed at 65 °C for 45 min. The amplicons were observed by the visible phenol red and the QIAxcel Advanced system (QIAGEN).

Mixture no.	Components and amount (μl)	LAMP result
1	male saliva 10 μl + female saliva 20 μl	+ve
2	semen 10 μl + female blood 20 μl	+ve
3	female blood 10 μl	-neg
4	semen 30 μl + female saliva 20 μl	+ve
5	male blood 10 μl + female blood 40 μl	+ve
6	semen 10 μl + female saliva 10 μl	+ve
7	male blood 10 μl + female saliva 10 μl	+ve
8	male saliva 20 μl + female saliva 10 μl	+ve
9	female saliva 10 μl	-neg
10	semen 10 μl + female blood 20 μl	+ve

had previously undergone AP and ME. The AP, ME, and LAMP results were compared and evaluated against the result of Y-STR profiling. Sensitivity, specificity, PPV, NPV, and ROC curves were applied for evaluation. The ROC curve was plotted for all methods to determine AUC percentage.

The AP test demonstrated the lowest value for PPV (46.7 %), specificity (43.9 %), NPV (78.1 %) and accuracy (57.6 %) (Fig. 3). In contrast, ME showed a higher value of PPV (100 %), specificity (100 %), NPV (75 %) and accuracy (79.3 %) (Fig. 3). However, ME also displayed the lowest sensitivity (45.7 %) among these comparisons. Remarkably, the LAMP technique exhibited the highest efficiency for all indexes with AUC value of 95.7 % (Fig. 3). To further evaluate the correlation between the results of the AP test, ME, and LAMP with the Y-STR profiling



Test	% AUC
LAMP	95.7
AP	61.9
ME	72.9

LAMP

	Y-STR (+)	Y-STR (-)	Total	Efficiency
LAMP (+)	32	0	32	Sensitivity 91.4% PPV 100% Specificity 100% NVP 75% Accuracy 79.3%
LAMP (-)	3	57	60	
Total	35	57	92	

Acid phosphatase

	Y-STR (+)	Y-STR (-)	Total	Efficiency
AP (+)	28	32	60	Sensitivity 80% PPV 46.7% Specificity 43.9% NVP 78.1% Accuracy 57.6%
AP (-)	7	25	32	
Total	35	57	92	

Microscopic examination

	Y-STR (+)	Y-STR (-)	Total	Efficiency
ME (+)	16	0	16	Sensitivity 45.7% PPV 100% Specificity 100% NVP 75% Accuracy 79.3%
ME (-)	19	57	76	
Total	35	57	92	

Fig. 3. Efficiency of LAMP, AP and ME compared to Y-STR in 92 sexual assault samples.

results, the correlation was assessed by the chi-square test. The results indicated that the AP test and ME showed no significant correlation with Y-STR profiling (Supplement Tables 2–3), whereas the LAMP technique exhibited a significant correlation with Y-STR profiling (**Supplement Table 4**).

3.6. Case study

Eight swab samples were collected from various areas and subjected to both AP and ME. DNA was isolated from the residual swab samples, followed by LAMP testing and Y-STR profiling. The results of each method were compared to the Y-STR profiling results (Supplement Table 5). The swab samples showed a DNA concentration ranging from 0.54 to 2.22 ng/μl, with varying numbers of typable Y-STR loci observed, spanning from a single locus to a complete 25 Y-STR loci.

Among the endocervical samples, two displayed positive AP test results and one of these samples aligned with the LAMP and Y-STR results. Conversely, ME yielded negative results for all the samples. However, the LAMP test provided positive results for four out of the eight samples. Remarkably, these four samples exhibited 4, 8, 16 and 25 typable Y-STR loci, respectively.

4. Discussion

The development of a sensitive and specific screening test for male DNA that can be performed at the crime scene is highly desirable in forensic investigations. LAMP, a novel amplification technique, has shown promising results for this purpose [11–13]. In this study, we designed a set of LAMP primers, including loop primers, specifically targeting the AMELY gene. Positive results were consistently obtained only in the presence of male DNA, demonstrating the specificity of the assay across all detection methods. The LAMP technique's main advantages lie in its high sensitivity and rapidity. Most studies have incorporated loop primers in the assay to accelerate the exponential amplification in a shorter time [11,13,18]. We conducted the LAMP assay at two different incubation times: 30 min and 45 min. Remarkably, at 30 min, the LAMP assay consistently produced positive results for as low as 0.1 ng of male DNA (Fig. 2), which is consistent with the sensitivity of other studies [11,13]. In one replicate, intermediate coloration was observed when the DNA input was 0.001 ng. This phenomenon later transitioned into a positive result after extending the reaction time by an additional 15 min. When DNA polymerase added a new dNTP, it releases a by-product proton ion, resulting in a lower pH reaction that affects the color of Phenol red. The limited quantity of DNA templates may require a longer incubation time to reach the yellowish color of Phenol red [21]. The choice of detection platform depends on the specific purpose of the study. Detection methods can range from simple turbidity or colorimetry to more advanced techniques such as qPCR, on-chip assays, or lateral flow assays. Each detection platform has its own unique advantages and limitations [10]. At present, Kitamura and colleagues [12] showed the highest sensitivity for male DNA detection using LAMP technique. Their assay can detect as little as 1 pg of DNA template using fluorescent signal monitoring. They designed LAMP primers targeting alphoid repeat regions, which consist of repetitive sequences [22], potentially increasing the target sequences for the LAMP assay. The fluorescent dye coupling real-time PCR was used to detect LAMP product in optimization and validation studies. The combination of a multi-copy target and a fluorescent dye with real-time PCR may contribute to the high sensitivity of the test.

Azoospermia is where a small number of spermatozoa or none is detected in the semen and thus this type of specimen is presumably challenging for AP test, ME and LAMP assay. Regardless of its unmeasurable spermatozoa, AP test and ME were able to verify the trace of AP and spermatozoa, respectively. Negative result was observed from LAMP assay. This may be due to the DNA input (0.08 ng) that is below the sensitivity of this test (0.1 ng). Increasing DNA input (more than 2 μl) may accelerate the reaction to produce positive result.

For species specificity, primates are the most potential cross-reactivity with amelogenin-based LAMP assay. The close evolution between human and primates results in high similarity in genetic sequences. In this study, *Macaca fuscata*, *Macaca fascicularis* and *Macaca mulatta* are chosen to test since these species commonly found in Asia. *Pongo pygmaeu* and *Pan troglodytes* are chosen to represent the apes. The results indicated that Macaca genus and *Pan troglodytes* were able to produce false positive in LAMP assay regardless of its gender. Furthermore, LAMP assay can differentiate gender in *Pan troglodytes*.

To validate the compatibility of the LAMP assay with forensic samples, we employed the assay to detect male DNA in mock and casework samples. The LAMP assay accurately identified all mock samples containing male biological fluid. This also reflected the efficiency of the Prepfiler™ Forensic DNA extraction kit (Applied Biosystems) in DNA extraction from mixtures of various biological fluids including semen. However, it is important to acknowledge that using the Prepfiler™ kit involves multiple steps and requires appropriate laboratory equipment.

To address these challenges, some recent studies have simplified DNA collection methods using crude lysate sample preparation [12,13]. For future study, we may explore the implementing crude lysate instead of extracted DNA to further simplify the process. This modification could offer a more practical and field-friendly approach, making the LAMP assay even more suitable for on-site applications in forensic investigations.

Despite their limitations, AP and ME remain commonly employed for semen detection due to their affordability. In this study, AP demonstrated the lowest efficiency, while ME exhibited high specificity but low sensitivity (Fig. 3). These findings are consistent with expectations when different methodologies have been compared. AP is to identify the presence of AP enzyme in the specimen and therefore should not be considered along with LAMP assay or Y-STR profiling. While AP should not be directly compared to LAMP assays or Y-STR profiling, the results nevertheless highlight the relative efficacy of each test for forensic investigations. Out of 92 casework samples, the LAMP assay yielded concordant results with Y-STR in all but three samples. In these discrepant cases, LAMP yielded negative results, but other tests produced different outcomes. The low amount of male DNA was presumed to be the reason in two samples, as indicated by Y-STR profiling, which generated 2 and 8 typable loci out of 25, respectively. The other casework sample displayed a negative for LAMP result in contrast to the complete profile of Y-STR. This discrepancy could be attributed to an inhibitor in the sample that affects LAMP, but not Y-STR. Similar findings were observed in the case study, where samples were collected from different locations (Supplement Table 5). According to the Yfiler™ Plus's validation studies [23], full Y-STR profile can be obtained from DNA input in the range of 0.125–1.000 ng. This DNA input range conforms to the LAMP assay's DNA input range (0.100–1.000 ng). However, when DNA input is less than 0.1 ng, the LAMP assay would show negative result for male DNA. This may result in restraining from downstream DNA analysis or Y-STR genotyping may show partial profile. It is a gap in this study and required further investigations to resolve this issue. Furthermore, there was a high correlation between the LAMP assay and Y-STR, indicating that the LAMP has the potential to predict Y-STR outcomes, thereby streamlining downstream analyses. The future study will incorporate Y-specific qPCR to accurately determine the true quantity of male DNA in the sample. The comparison of qPCR, LAMP assay and Y-STR efficiencies would be more appropriate since these tests are specifically designed to detect male DNA.

5. Conclusion

In conclusion, the development and evaluation of the colorimetric LAMP assay have shown promising results for male DNA detection. Its high sensitivity and accessibility make it a valuable tool for forensic investigations, potentially expediting and enhancing the identification of male DNA in various forensic samples. Future studies may focus on elucidating the impact of inhibitors on the LAMP assay and refining the LAMP detection limit, to enable Y-STR profile generation. Moreover, investigations with a larger sample sizes and more diverse types of forensic samples are warranted to strengthen the evidence for the LAMP test's practical applicability in forensic casework.

CRediT authorship contribution statement

Sunita Chunkul: Writing – original draft, Methodology, Investigation. **Tikumphorn Sathirapatya:** Writing – review & editing, Writing – original draft. **Piyawan Dangklao:** Writing – review & editing. **Praphat Kawicha:** Writing – review & editing, Methodology. **Rachaneekorn Tammachote:** Writing – review & editing, Supervision, Conceptualization. **Kornkiat Vongpaisarnsin:** Writing – review & editing, Supervision, Conceptualization.

Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declaration of competing interest

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsisyn.2024.100567.

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