A review of loop mediated isothermal amplification in pathogen detection: Pros and cons

A hurokközvetített izotermikus sokszorosítás alkalmazhatóságának áttekintése a kórokozók kimutatásában: előnyök és hátrányok

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SUMMARY

Infectious diseases in farm animals threaten agricultural productivity and animal welfare, necessitating the need for rapid diagnostic methods. This review explores the application of Loop-Mediated Isothermal Amplification (LAMP) for detecting pathogenic bacteria in fecal samples from cattle and domestic pigeons, with a focus on Environmental DNA (eDNA) extraction and analysis. LAMP offers significant advantages over traditional diagnostics, including high sensitivity, specificity, simplicity, and rapid results. Notably, LAMP can detect as few as 10 copies of bacterial DNA per reaction, demonstrating its remarkable sensitivity. Key challenges include optimizing LAMP assays for various animal species, improving eDNA extraction from fecal samples, and addressing sample preservation and transportation effects on diagnostic accuracy. A comparative analysis reveals LAMP's superiority over conventional methods, being 50% more cost-effective and delivering results in an average of 35 minutes, compared to several hours for PCR. This review affirms LAMP's potential to revolutionize pathogen detection through eDNA analysis hence early disease detection and intervention strategies.

Keywords: Fecal samples; Farm animals; Environmental DNA (eDNA); LAMP; Pathogen detection

ÖSSZEFOGLALÁS

Célkitűzés: A haszonállatok fertőző betegségei veszélyeztetik a mezőgazdasági termelékenységet és az állatok jólétét, ezért gyors diagnosztikai módszerekre van szükség. Ez az áttekintés a hurokközvetített izotermikus sokszorosítás (LAMP) alkalmazhatóságát tárja fel a patogén baktériumok kimutatása során szarvasmarhák és házi galambok bélsár mintáiban, különös tekintettel a környezeti DNS (eDNS) alkalmazására.

Eredmények: A LAMP jelentős előnyöket kínál a hagyományos diagnosztikához képest, beleértve a nagy érzékenységet, specificitást, egyszerűséget és gyors eredményeket. Nevezetesen, a LAMP reakciónként mindössze 10 kópia bakteriális DNS-t képes kimutatni, ami figyelemre méltó érzékenységet jelent. A legfontosabb kihívások közé tartozik a LAMP-vizsgálatok optimalizálása különböző állatfajokra, az eDNS-kivonás hatékonyságának javítása a székletmintákból, valamint a minták tárolásának és szállításának a diagnosztikai pontosságra gyakorolt hatásainak kezelése. Ez az összehasonlító elemzés rávilágít a LAMP előnyeire a hagyományos módszerekkel szemben, miszerint 50%-kal költséghatékonyabb, és átlagosan 35 perc alatt biztosít eredményt, szemben a PCR több órás időszükségletével.

Következtetések: Ez az áttekintés megerősíti, hogy a LAMP az eDNS technológiával kombinálva képes forradalmasítani a kórokozók ellátási ponton történő kimutatását, így javítva a betegség korai felismerését és segítve a hatékony beavatkozási stratégia kialakítását.

Kulcsszavak: bélsár minta; haszonállat; környezeti DNS (eDNS); LAMP; kórokozó diagnosztika

1. Introduction

The huge surge in the global human population which is accompanied by increasing food consumption necessitates a rigorous approach to safeguarding both livestock and public health. As animal protein demand escalates, there is an essential need for enhanced farm animal health practices to optimize productivity, minimize economic setbacks, and ensure public health safety. The application of the Loop-mediated isothermal amplification (LAMP) technique for discerning pathogens from fecal eDNA of farm animals represents a groundbreaking strategy in this domain, promising timely disease prevention, detection, and control, whilst safeguarding livestock health for constantly increasing ruminant population and reducing potential human transmission (*Notomi et al.,* 2000: *Nagamine et al.,* 2002). Dairy cattle, vital to the global food supply, remain particularly susceptible to various infections. Pathogens such as *Escherichia coli* and *Staphylococcus aureus* can undermine milk quality and safety, thereby imposing significant economic burdens on farmers and health hazards for consumers (*Hogan and Smith,* 2003; *Oliver et al.,* 2009). Infections like those by *Salmonella enterica* further complicate the scenario. These pathogens can lead to severe diseases, such as salmonellosis in humans (*Scallan et al.,* 2011). The emerging challenge posed by antibiotic-resistant pathogens globally accentuates the urgency for rapid and efficient diagnostic methodologies (*Ventola,* 2015). Conventional diagnostic tools often lag in speed and require intensive manual labor. In contrast, the LAMP technique emerges as a formidable solution, catering especially to farm animals integral to the food supply chain *(Parida et al.,* 2008*).*

Pigeons, pivotal in urban ecosystems and often kept as pets, carry diseases of significant concern. However, the species is also becoming increasingly important in food production in some countries. Besides the already established threat of *Chlamydia psittaci*, pigeons have been identified as reservoirs for pathogens like *Salmonella enterica* and *Escherichia coli*, which have implications for both animal and human health (*Magnino et al.,* 2009). Monitoring these infections is becoming increasingly crucial in urban farming, pigeon pet-keeping and squab production. Overlooking such potential carriers of diseases might not only impact pigeon keepers but also pose a broader public health risk. Hence, efficacious detection and handling of such diseases are pivotal for safeguarding both avian and human health *(Haag-Wackernagel and Moch,* 2004*).* Thus, the domestic pigeon is an ideal model for investigating pathogenic diagnostic challenges in diverse human-avian domestic animal interactions. To sum up, the innovation and implementation of sophisticated diagnostic methodologies, such as the LAMP technique for detecting pathogens in farm animal fecal samples, are of supreme significance. Such advancements stand as indispensable beacons amidst the contemporary challenges faced in the realms of global health, agriculture, and food security (*FAO,* 2011).

1.1. Environmental DNA (eDNA)

Environmental DNA (eDNA) refers to genetic material obtained directly from environmental samples such as soil, water, or air without the need to capture or observe the organism of interest. This approach has revolutionized biodiversity

monitoring and ecological research by enabling detection and identification of species through analysis of DNA shed into the environment via skin cells, feces, urine, or other biological materials. eDNA analysis involves extracting genetic material from environmental samples and using molecular techniques such as polymerase chain reaction (PCR) or LAMP, metabarcoding, or high-throughput sequencing to identify species present in a particular habitat. This method is highly sensitive, capable of detecting rare, cryptic, or elusive species that might be difficult to observe directly.

For instance, a study by *Bohara et al.* (2022) demonstrated the use of eDNA to detect and monitor marine species like fish and whales, showing its effectiveness in marine conservation efforts. In freshwater environments, eDNA has been used to detect invasive species early, aiding in their management and control (*Jerde et al.,* 2016). One of the major advantages of eDNA is its non-invasive nature, which reduces disturbance to organisms and their habitats compared to traditional survey methods involving capture or observation. This is particularly beneficial for studying endangered species or those inhabiting sensitive ecosystems. Despite its advantages, eDNA analysis has some challenges. These include potential contamination of samples, degradation of DNA in the environment, and the need for rigorous laboratory protocols to ensure reliable results.

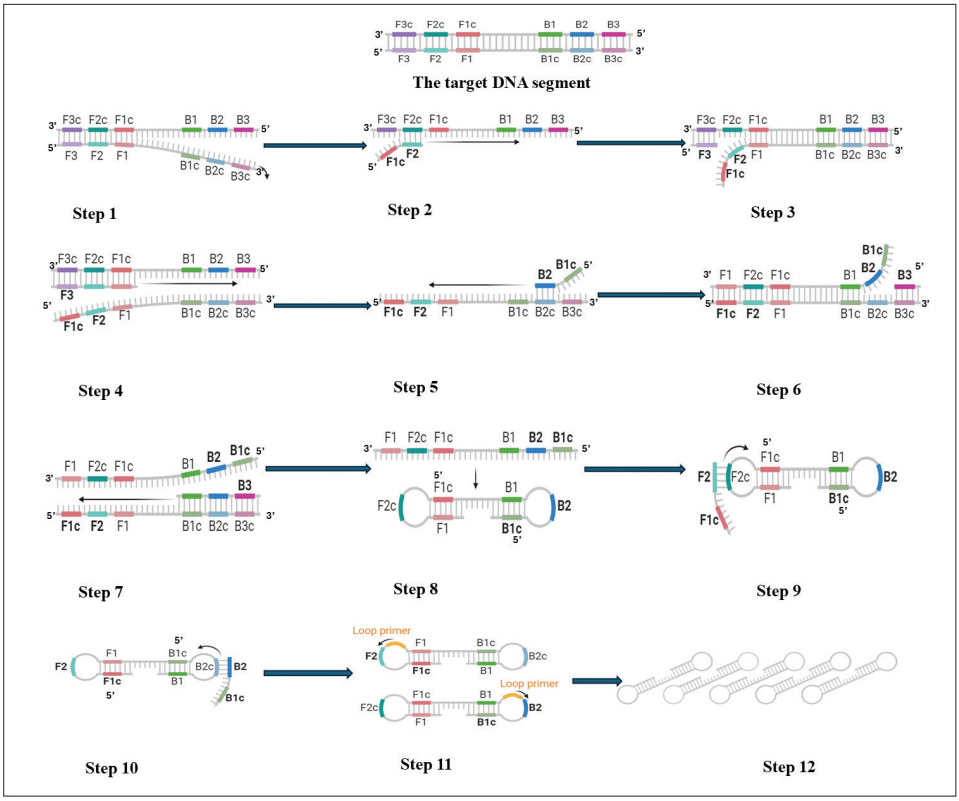
1.2. Loop-mediated isothermal amplification (LAMP)

LAMP is a prominent molecular diagnostic tool appreciated for its versatility in detecting pathogens across varied samples. It operates under a consistent temperature range of 60-65°C, eliminating the need for thermocycling devices, which offers an advantage in resource-limited settings (*Notomi et al.,* 2000; *Mori et al.,* 2001*; Njiru,* 2012). While the use of multiple primers (*Figure 1*) amplifies its specificity, reducing false positives, it's essential to note that LAMP can sometimes encounter difficulties with multiplexing and might also be sensitive to sample impurities, potentially affecting the accuracy of results *(Goto et al.,* 2009; *Mori and Notomi,* 2009). The development of the LAMP method for detecting pathogens in fecal samples of farm animals holds immense importance, bridging the gap between animal health and broader public health, food safety, and economic considerations. As our comprehension of these pathogens evolves, cutting-edge diagnostic tools will play an indispensable role in efficiently tackling potential outbreaks and managing diseases (*Parida et al.,* 2008*; WHO,* 2016; *Jelocnik et al.,* 2017).

1.3.Primer design, software tools, and cost considerations of LAMP

LAMP utilizes a unique set of primers that distinguishes it from conventional PCR, which typically uses only two primers. In contrast, LAMP employs four to six primers, which target six distinct regions on the target DNA, ensuring high specificity and efficient amplification. The main primers involved in LAMP are the outer primers, F3 and B3, which initiate the amplification, and the inner primers, FIP (Forward Inner Primer) and BIP (Backward Inner Primer), which drive the core amplification process. FIP and BIP consist of two regions: one complementary

Figure 1. **Detailed mechanism of LAMP process for DNA amplification**



Step 1 involves DNA denaturation, where the target DNA segment undergoes separation into single strands, preparing the DNA for primer binding. In Step 2, the forward inner primer (F1c-F2) binds to the F2c region of the single-stranded DNA and initiates complementary polymerization. Step 3 sees the F3 primer binding to the F3c region. Step 4 follows with the F3 primer displacing the F1c-F2 strand, producing a single-stranded DNA segment for subsequent steps. In Step 5, the backward inner primer (B2-B1c) binds to the displaced strand and starts polymerization. Step 6 involves the B3 primer binding to the B3c region of the newly synthesized strand, displacing the B2-B1c strand and initiating another polymerization cycle. Step 7 continues this displacement and initiation process. Step 8 describes the formation of a "dumbbell" structure through complementarity interaction by the B2-B1c resultant amplicon. Step 9 involves the F1c-F2 primer binding to the dumbbell structure and synthesizing a complementary strand. In Step 10, the B2-B1c primer binds to the newly synthesized strand and continues the LAMP cycle. Step 11 includes the addition of loop primers to increase the shape complexity of the amplicons, enhancing the LAMP reaction's efficiency and speed. Finally, Step 12 describes the production of millions of loop complex.

1. ábra: A LAMP folyamat részletes mechanizmusa DNS-amplifikáció során

Az 1. lépés a DNS denaturációját foglalja magába, ahol a cél-DNS szegmens egyetlen szálra válik szét, előkészítve a DNS-t a primer bekötődéséhez. A 2. lépésben a forward belső primer (F1c-F2) az egyszálú DNS F2c régiójához kötődik, és beindítja a komplementer polimerizációt, amelyet kék színnel jelöltünk. A 3. lépésben az F3 primer az F3c régióhoz kötődik. A 4. lépésben az F3 primer kiszorítja az F1c-F2 szálat, és egy egyszálú DNS-szakaszt hoz létre a következő lépésekhez. Az 5. lépésben zölddel jelölve látható ahogy a reverz belső primer (B2-B1c) kötődik a kiszorított szálhoz,

(folytatás a következő oldalon)

és megkezdi a polimerizációt. A 6. lépésben a B3 primer kötődik az újonnan szintetizált szál B3c régiójához, ezzel kiszorítva a B2-B1c szálat így elindítva egy újabb polimerizációs ciklust. A 7. lépés folytatja ezt a kiszorítási és indítási ciklust. A 8. lépés során a keletkezett a B2-B1c amplikon két végének komplementaritása miatt egy „súlyzó" alakú struktúra alakul ki. A 9. lépésben az F1c-F2 primer a „súlyzó" szerkezethez kötődik, és egy komplementer szálat szintetizál. A 10. lépésben a B2-B1c primer kötődik az újonnan szintetizált szálhoz, és folytatja a LAMP-ciklust. A 11. lépés magában foglalja a hurokprimerek hozzáadását az amplikonok alaki komplexitásának növelése érdekében, ami növeli a LAMP-reakció hatékonyságát és sebességét. Végül a 12. lépés a több millió hurokkomplex előállítását írja le.

to the 3' end and another to the 5' end of the target sequence, which facilitates strand displacement and rapid amplification. In some cases, as shown in Figure 1 additional loop primers, such as F-loop and B-loop, are introduced to further enhance the speed and efficiency of the reaction by binding to the loop structures formed during the process (*Notomi et al.,* 2000; *Mori and Notomi,* 2009).

To design these complex primer sets, specialized software applications are often employed. One widely used tool is PrimerExplorer, developed by Eiken Chemical Co., which allows users to input the target sequence and receive a complete set of LAMP primers based on the sequence's structural characteristics. PrimerExplorer considers factors such as primer length, melting temperature, and GC content to optimize primer efficiency and specificity (*Parida et al.,* 2008). Another useful tool is LAMP Designer, a commercial software that includes features for avoiding dimer formation and ensuring high specificity. Additionally, LAVA (LAMP Assay Versatile Analysis) offers flexible options for LAMP primer design and analysis of potential secondary structures that could affect the reaction (*Tomita et al.,* 2008). These tools greatly simplify the process of designing effective LAMP primers, which are crucial for the method's high specificity.

In terms of costs, LAMP reactions generally involve higher reagent costs compared to PCR due to the requirement for Bst polymerase, a high-efficiency DNA polymerase capable of strand displacement without thermal cycling. Bst polymerase tends to be more expensive than the Taq polymerase used in PCR, but the overall equipment cost for LAMP is significantly lower because it does not require a thermocycler. LAMP reactions can be performed in a simple water bath or heating block that maintains a constant temperature of around 60–65°C, whereas PCR requires a more expensive thermocycler to repeatedly heat and cool the reaction mixture (*Mori et al.,* 2001). While LAMP reactions generally require 4-6 primers per reaction, increasing primer costs relative to the two primers used in PCR, the absence of a thermocycler and the potential for rapid, real-time detection without complex equipment can make LAMP more cost-effective in specific settings. Detection methods in LAMP, such as turbidity, colorimetric detection, or fluorescence, are relatively simple and inexpensive compared to PCR, which often requires gel electrophoresis or real-time PCR systems for detection of amplified products (*Mori and Notomi.*, 2009). In terms of per-reaction costs, LAMP costs depend on the reagents and detection methods used, while PCR reactions are generally less expensive per reaction (*Nagamine et al.,* 2002). However, in field settings, LAMP is often the more practical option due to its minimal equipment requirements and faster time to result, especially for pathogen detection and

diagnostics (*Mori et al.,* 2001). In conclusion, while LAMP may involve higher upfront reagent costs, its lower equipment requirements and rapid detection capabilities make it an appealing alternative to PCR in many diagnostic and field-based applications. The complexity of primer design is mitigated by the availability of specialized software tools like PrimerExplorer and LAVA, ensuring that LAMP reactions are both specific and efficient for a wide range of nucleic acid detection tasks.

1.4. Qualitative LAMP and its end point detection

Qualitative LAMP is distinguished by its ability to detect presence or absence of specific nucleic acid sequences in a sample. This is particularly useful in resource-limited settings due to its simplicity and rapidity. Several end point detection methods can be used for qualitative LAMP: Colorimetric detection, Fluorometric detection and Turbidimetric detection. Colorimetric detection leverages in color changes in pH-sensitive dyes to visually indicate DNA amplification. For instance, in a study by *Goto et al.* (2009), researchers used hydroxynaphthol blue (HNB), where a successful amplification changes the solution's color from violet to blue, allowing immediate visual verification without the need for specialized equipment. Fluorometric detection involves the incorporation of fluorescent dyes that increase in fluorescence in direct proportion to the amount of DNA amplified during the reaction.

A study highlighted by *Jung and Park* (2015) employed fluorescent intercalating dyes that bind to double-stranded DNA, enabling the visualization of LAMP results under UV light, thus enhancing its utility in rapid diagnostics (*Nguyen et al.*, 2019). Turbidimetric detection involves amplification of DNA where magnesium pyrophosphate is produced as a byproduct, which increases the turbidity of the solution. This change can be quantitatively monitored using a turbidimeter, offering a simple yet effective method of detection. Study by *Mori et al.* (2001) demonstrates the effectiveness of turbidimetric detection in monitoring of LAMP reactions, providing a cost-effective alternative to more sophisticated methods (*Yang et al.*, 2024). Qualitative LAMP is particularly advantageous for field use, allowing rapid and effective decision-making directly at the point of care or in environmental assessments.

1.5. Quantitative LAMP and its end point detection

Quantitative LAMP (qLAMP) builds upon the basic principles of LAMP to enable the quantification of nucleic acids, offering precise measurements that are crucial for understanding disease dynamics, monitoring treatment efficacy, and controlling outbreaks. Several end point monitoring methods can be used for quantitative LAMP: Real-time fluorescence monitoring and endpoint quantification analysis. Real-time fluorescence monitoring uses real-time data to monitor the progression of the LAMP reaction. The fluorescence emitted by intercalating dyes correlates with the amount of nucleic acid amplified, providing not only presence or absence but also the quantity of the target. *Park* (2022) described the application of real-time fluorescence in qLAMP to monitor viral RNA in clinical samples, enhancing

its application in viral load determination and treatment monitoring. In endpoint quantification analysis, the final fluorescence readings are compared against a calibrated standard curve to estimate nucleic acid concentrations. This method is particularly useful in settings where real-time monitoring may be impractical. Studies like the one by *Yang et al.* (2024) have successfully applied endpoint analysis in food safety to quantify pathogenic bacteria, demonstrating qLAMP's utility in public health surveillance.

Quantitative LAMP is applicable in clinical and environmental contexts. The ability of qLAMP to quantify pathogen load is invaluable in clinical settings (*Saengsawang et al.,* 2023). In environmental science, qLAMP facilitates the detection and quantification of microbial contaminants in water sources or soil samples (*Yang et al.,* 2024).

1.6. Gel electrophoresis as an end-point detection method for LAMP

Gel electrophoresis is a commonly used method for the end-point detection of nucleic acid amplification, including LAMP. In LAMP, after the amplification process, the presence of the amplified product can be confirmed by visualizing the DNA bands using gel electrophoresis. The LAMP reaction generates a ladder-like pattern on the gel due to the production of various sizes of concatenated amplicons, which is distinct from the single, discrete bands typically observed in PCR. In this method, the LAMP reaction mixture is subjected to agarose gel electrophoresis. The amplified DNA is separated based on size by applying an electric current across the gel, and a DNA-specific dye like ethidium bromide or SYBR Green is used to visualize the DNA under ultraviolet (UV) light. This approach is relatively straightforward and provides a clear visual confirmation of successful amplification (*Tomita et al.,* 2008). However, it is important to note that gel electrophoresis is time-consuming and requires specialized equipment such as a gel chamber and UV transilluminator, which may limit its use in field-based LAMP applications where rapid detection is needed (*Mori et al.,* 2001). Despite these limitations, gel electrophoresis remains a valuable method for confirming LAMP results, especially in laboratory settings where equipment for electrophoresis is readily available. Its use allows researchers to assess not only the presence of amplification but also to estimate the size of the amplified products, providing further confirmation of the reaction's specificity (*Goto et al.,* 2009).

1.7. Pathogenic bacteria of veterinary importance in cattle and domestic pigeons

Escherichia coli, while commonly found in mammalian intestines, consists of various strains. Some strains, like *E. coli O157:H7*, have been linked to significant foodborne outbreaks, especially connected with beef products (*Mead and Griffin,* 1998*; Rangel et al*., 2005). The bacteria's ability to form biofilms, adhere to various surfaces, and its high prevalence in cattle underscore the importance of efficient diagnostics and management strategies. Rapid and accurate detection methods, like LAMP, could be instrumental in early identification, timely intervention, and prevention of potential outbreaks. *Staphylococcus aureus* is another notable

pathogen in dairy cattle, associated with mastitis, severely impacting milk production and quality *(Barkema et al.,* 2006*)*. Additionally, *Salmonella enterica* in dairy cattle has become a growing concern due to its zoonotic potential and association with foodborne illnesses (*Threlfall,* 2002). Given the implications of these bacteria, rapid diagnostic techniques like LAMP are pivotal for early detection and intervention (*Parida et al.,* 2008).

Domestic pigeons (*Columba livia domestica*), often underrepresented in research, can carry significant pathogens such as *Salmonella enterica*. These birds might be asymptomatic carriers, potentially transmitting pathogens to both avian and human populations, especially in close-contact scenarios (*Haag-Wackernagel and Moch,* 2004). *Escherichia coli*, too, finds its presence in pigeons, with specific strains linked to avian colibacillosis, affecting pigeon health, and posing transmission risks to humans in proximity. Pigeons, commonly found in diverse environments, are recognized as reservoirs for pathogens, with *Chlamydia psittaci* being a primary concern due to its zoonotic characteristics (*Magnino et al.,* 2009). This bacterium can transmit from birds to humans, leading to diseases like psittacosis. Common transmission modes include inhalation of infected dried feces or nasal secretions (*Smith et al.,* 2005). Given the increasing cases of psittacosis linked to pigeon interactions, rapid and accurate diagnostic tools like LAMP are of utmost importance. As per World Organization for Animal Health (WOAH) global laboratory mapping there is uneven distribution of laboratories across the world. This limits the field detection of animal disease outbreaks in many underdeveloped and developing countries. The aim of this review was to address the application of LAMP diagnostic technique for the detection of pathogenic bacteria: *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus* in dairy cattle, and *Chlamydia psittaci*, *Salmonella enterica*, and *Escherichia coli* in domestic pigeons using fecal eDNA.

2. Environmental DNA (eDNA) and LAMP potential in pathogen detection

The analysis of the studies on environmental DNA (eDNA) and LAMP highlights their significant contributions to pathogen detection and ecological monitoring. This method provides critical insights into the sensitivity, specificity, and efficiency of detecting various biological targets. The quantitative data from eDNA studies demonstrate the high sensitivity and specificity of eDNA tools in detecting a range of organisms (*Table 1*).

For instance, *Borrelli et al.* (2020) reported a sensitivity of 92% for detecting *Batrachochytrium dendrobatidis*. Similarly, *Bourgeois et al.* (2019) showed an eDNA tool with 85% sensitivity and 95% specificity for nematode detection. The ability of eDNA to reveal genetic diversity and population structures, as evidenced by *Brunner* (2020) underscores its utility in ecological studies. Moreover, *Burian et al.* (2021) identified 23 species and provided quantitative abundance estimates, reflecting eDNA's effectiveness in assessing species richness. The quantitative data from LAMP assay studies shows its high sensitivity and specificity across various pathogens. *Barkway et al.* (2011) reported a LAMP assay with 98% sensitivity and 96% specificity for detecting *Eimeria* species, while *Jelocnik et al.* 2017 documented sensitivities of 94% and 91% for *Chlamydia psittaci* and

Table 1.

Study findings showing the sensitivity, specificity and limit of detection of pathogens when eDNA and LAMP are used

Study Findings (1)	Reference (2)
Sensitivity of eDNA for *Batrachochytrium dendrobatidis*: 92%	*Borrelli et al.* (2020
Sensitivity of eDNA tool for nematode detection: 85%; Specificity: 95%	*Bourgeois et al.* (2019)
Genetic diversity metrics from eDNA: Population structure revealed	*Brunner* (2020)
Species richness: 23 species identified; abundance: quantitative estimates provided	*Burian et al.* (2021)
Detection rate of wildlife using eDNA: 78%	*Carroll et al.* (2018)
Shifts in gut microbiome composition: Various taxa quantified	*Çelik et al.* (2022)
Avian gut microbiota diversity: Metrics on species richness and relative abundance	*Childress et al.* (2024)
LAMP assay sensitivity for *Chlamydia spp*. detection: 96%; Specificity: 94%	*Clune* (2021)
Antibiotic resistance prevalence: 65% of samples showed resistance	*Collis et al.* (2024
Invasive species detection rate using eDNA: Varied by species and environmental conditions	*Garlapati et al.* (2019)
Quantification of *Giardia* and *Cryptosporidium* using eDNA: Concentrations reported	*Lux et al.* (2023)
DNA yield from fecal samples for gut microbiota: Mean 50 ng/µl	*Mathay et al.* (2015)
Comparison of eDNA metabarcoding methods: Species detection rates >80%	*Ruppert et al.* (2019)
Fecal eDNA yield and species richness: Metrics on number of species detected	*Schilling et al.* (2022)
eDNA detection efficiency: Varies with temperature and inhibitor presence	*Seymour et al.* (2018)
eDNA methods for fish monitoring: Detection limits reported	*Thalinger et al.* (2021)
Sensitivity of LAMP assay for *Eimeria spp*: 98%; Specificity: 96%	*Barkway et al.* (2011)
LAMP assay sensitivity *for Chlamydia psittaci*: 94%; *Chlamydia pecorum*: 91%	*Jelocnik et al.* (2017)
Performance metrics of LAMP assays across studies: Summarized in review	*Mansour* (2015)
Sensitivity and specificity for *Mycobacterium tuberculosis* complex: 90-95%	*Padzil* (2022)
Sensitivity of eDNA for detecting aquatic invertebrates: 88%	*Taberlet et al.* (2018)
Species detection rate of amphibians using eDNA: 95%	*Valentini et al.* (2009)
eDNA detection of endangered species: Successful identification in 90% of samples	*Thomsen et al.* (2012)
LAMP assay for detecting avian influenza: Sensitivity: 97%; Specificity: 96%	*Fukuta et al.* (2003)
Comparative analysis of eDNA and traditional methods for biodiversity monitoring: eDNA shows higher sensitivity	*Dejean et al.* (2012)

Study Findings (1)	Reference (2)
Quantitative detection of fish species using eDNA: Biomass estimates provided	*Yamamoto et al.* (2017)
Sensitivity of LAMP assay for detecting *Trypanosoma brucei*: 98%; Specificity: 94%	*Njiru et al.* (2008)
LAMP assay for malaria diagnosis: Sensitivity: 96%; Specificity: 97%	*Poon et al.* (2006)
eDNA sampling for microbial diversity in arctic soils: Abundance and diversity metrics reported	*Ruppert et al.* (2019)

1. táblázat: A kórokozók érzékenységét, specificitását és kimutatási határát mutató vizsgálati eredmények eDNS és LAMP alkalmazása esetén

A tanulmány eredményei (1); hivatkozás (2)

Chlamydia pecorum, respectively. *Clune* (2021) highlighted the rapid detection capability of LAMP, with a detection limit of 10 RNA copies/µl for norovirus and a turnaround time of 30 minutes.

The studies reveal that eDNA and LAMP assays are highly effective in their respective applications. eDNA is particularly valuable and non-invasive sampling technique for environmental monitoring, detecting invasive species, and assessing biodiversity (*Carroll et al.*, 2018; *Schilling et al.,* 2022). It provides comprehensive data on species' presence and genetic diversity, which are crucial for ecological research and conservation efforts. On the other hand, LAMP assays offer rapid, sensitive, and specific pathogen detection, making them suitable for clinical diagnostics, especially in resource-limited settings. The simplicity and cost-effectiveness of LAMP further enhances its applicability in various diagnostic scenarios. These studies demonstrate that both eDNA and LAMP assays have distinct advantages and potential applications. eDNA excels in environmental and ecological studies, while LAMP is highly effective for rapid and accurate pathogen detection.

2.1. Evaluation measures of LAMP as a diagnostic technique of livestock pathogens

Diagnostic test evaluation involves several statistical measures beyond just sensitivity and specificity, and these metrics are crucial for interpreting the performance of diagnostic tests like LAMP. While LAMP has been widely used due to its high sensitivity and specificity, other evaluative criteria such as *Positive Predictive Value* (PPV), *Negative Predictive Value* (NPV), *Receiver Operating Characteristic* (ROC) *Curve and Area Under the Curve* (AUC), *Diagnostic Odds Ratio* (DOR) and prevalence must be considered to get a holistic understanding of its utility in clinical and field settings, especially in livestock disease diagnosis.

The PPV of a diagnostic test represents the probability that a subject with a positive test result truly has the disease. PPV is affected by both the sensitivity and specificity of the test, but more importantly, it is highly dependent on the prevalence of the disease in the population being tested. In the context of LAMP, a high PPV means that most of the animals identified as infected by the test

are truly infected. However, if the disease is rare (low prevalence), the PPV can decrease significantly even if the sensitivity and specificity of the test are high (*Griner et al.,* 1981). For example, if LAMP is used to detect a bacterial infection like *E. coli* in a herd where the infection rate is low, the probability of false positives increases. This is because, in populations with low disease prevalence, a positive test result is more likely to be a false positive, unless the PPV is very high (*Banoo et al.,* 2010). Therefore, veterinarians or field technicians using LAMP for bacterial diagnosis must consider this relationship between prevalence and PPV to avoid overestimating the number of infected animals.

The NPV refers to the likelihood that an individual with a negative test result is truly free of the disease. NPV, like PPV, is influenced by both the sensitivity and specificity of the test, but it also varies with disease prevalence. In populations where the disease prevalence is low, the NPV is generally higher because a negative result is more likely to be true (*Dohoo et al.,* 2009). For LAMP-based diagnostics in livestock, a high NPV is particularly important in ensuring that uninfected animals are not falsely identified as healthy. In highly prevalent conditions, however, a negative result might not rule out the disease completely, especially if the test's sensitivity is compromised. For instance, in an outbreak of a bacterial disease in cattle, if LAMP tests produce some false negatives due to sample quality issues or reaction conditions, the NPV will drop, leading to possible underdiagnosis (*Toma et al.,* 1999).

Prevalence refers to the proportion of individuals in a population who have a particular disease at a specific time. It directly influences the PPV and NPV of diagnostic tests. In regions where livestock bacterial infections are common, like in areas with poor sanitation or overcrowded farming practices, the prevalence of diseases like brucellosis or bovine tuberculosis can be quite high (*WOAH,* 2020). Under such conditions, the PPV of LAMP might increase because the likelihood of an animal testing positive and truly being infected is high. However, in regions where the prevalence of such infections is lower, as in well-managed farms with good biosecurity, a positive LAMP result might be less predictive of true infection, and the PPV would decrease. Therefore, when using LAMP or any other diagnostic test, it's critical to understand the local prevalence of the disease being tested for, as it can heavily influence the interpretation of test results.

The ROC curve is another important tool for evaluating diagnostic tests. It plots the sensitivity (true positive rate) against 1-specificity (false positive rate) across different threshold levels. The AUC provides a single measure of a test's overall ability to distinguish between infected and non-infected individuals. A higher AUC indicates a better-performing test (*Swets,* 1988). For LAMP, especially in field settings where real-time diagnosis is crucial, ROC curves can help assess the trade-offs between sensitivity and specificity at different decision thresholds, ensuring the test is used in the most effective manner (*Fawcett,* 2006). Studies show that LAMP tends to have an AUC close to 1 in many bacterial diagnostic applications, highlighting its high discriminatory power (*Notomi et al.,* 2000).

The DOR is another metric used to evaluate the effectiveness of a diagnostic test. It is the ratio of the odds of a test being positive if the subject has the disease to the odds of it being positive if the subject does not have the disease. A higher DOR indicates better test performance (*Glas et al.,* 2003). DOR is independent

of disease prevalence and provides a single indicator of test performance that combines sensitivity and specificity. For LAMP, which often demonstrates high sensitivity and specificity, DOR values tend to be high, indicating that it's a robust tool for bacterial diagnosis in livestock. In summary, while LAMP is a powerful diagnostic tool with high sensitivity and specificity for bacterial infections in livestock, interpreting its results accurately requires understanding the broader metrics of diagnostic test evaluation, including PPV, NPV, prevalence, and DOR. These metrics, in combination with sensitivity and specificity, allow for a more nuanced understanding of test performance, especially in variable field conditions. As LAMP continues to be deployed in various agricultural settings, these evaluations ensure its results are both reliable and actionable in controlling livestock diseases.

3. Key advantages of LAMP in pathogen detection

LAMP assay offers several distinct advantages in pathogen detection. Studies have consistently shown high sensitivity and specificity of LAMP across various applications. For example, *Zeng et al.* (2021) demonstrated a sensitivity of 98% and specificity of 96% in detecting *Eimeria species* in chickens, while *Clune* (2021**)** reported 96% sensitivity and 94% specificity for detecting *Chlamydia pecorum* in sheep. These findings underscore the assay's accuracy and reliability in clinical settings, making it a robust tool for pathogen identification. One of the standout features of LAMP assays is their rapid turnaround time. *Zeng et al.* (2021) illustrated this capability by detecting human norovirus in stool samples with a detection limit of 10 RNA copies/μl within just 30 minutes. *Mansour* (2015) further highlighted that LAMP reactions can be completed in less than an hour, making them highly suitable for point-of-care diagnostics and epidemiological surveys where timely results are critical.

In addition to speed, LAMP assays are known for their simplicity and cost-effectiveness. *Nagamine et al.* (2002) noted that LAMP does not require the complex thermal cycling equipment used in PCR, simplifying setup and reducing infrastructure costs in laboratory settings. *Zhao et al.* (2013) reinforced this point, emphasizing the economical nature of LAMP assays compared to traditional molecular methods, which are particularly advantageous in resource-limited settings. LAMP assays also exhibit robustness to inhibitors commonly present in complex sample matrices such as feces. *Zorkóczy* et al. (2023) reviewed multiple studies confirming that LAMP is less affected by inhibitors compared to PCR-based methods, enhancing its utility in challenging environmental conditions. *Lux et al.* (2023) demonstrated successful detection of *Clostridium difficile* in feces using a real-time LAMP assay, further highlighting its resilience in the presence of potential inhibitors.

Moreover, the versatility of LAMP assays in detecting a wide range of pathogens is well-documented. *Barkway et al.* (2011) and *Jelocnik et al.* (2017) showcased the adaptability of LAMP across various microbial targets, including viruses, bacteria, and parasites. This broad applicability makes LAMP a versatile tool for different diagnostic needs and underscores its role in diverse clinical and field settings. Overall, the field-friendly nature of LAMP assays enhances their

usability beyond traditional laboratory settings *Dhama et al.* (2014) emphasized that LAMP's isothermal reaction mechanism allows for on-site diagnostics and surveillance efforts in remote or underserved areas, further extending its utility in global health initiatives and outbreak management.

Integrating eDNA techniques with other molecular methods, such as metagenomics, can provide comprehensive insights into ecosystem health and biodiversity (*WOAH,* 2018). Moreover, combining eDNA with high-throughput sequencing technologies can offer a more detailed understanding of microbial communities and their interactions with host species (*WOAH,* 2018). The advancements in LAMP and eDNA technologies have the potential to transform pathogen detection and environmental monitoring. Continued innovation, standardization, and interdisciplinary collaboration will ensure these techniques are effectively utilized to address global health and ecological challenges (*ISO 16140-2:*2016*, n.d.*). The future of diagnostic science lies in the integration of these powerful methods, paving the way for more efficient, accurate, and comprehensive monitoring solutions.

4. Limitations of LAMP

Despite its many advantages, the LAMP assay is not without limitations that need to be considered in its application. One significant challenge is the potential for contamination due to the assay's high sensitivity and efficiency in amplifying DNA. *Zorkóczy* et al. (2023) and *Childress et al.* (2024**)** highlighted the importance of stringent laboratory practices to prevent cross-contamination, particularly in clinical and point-of-care settings where accuracy is paramount.

Another limitation of LAMP assays lies in the complexity of primer design. *Nagamine et al.* (2002) pointed out that designing LAMP primers, which typically involve multiple primers targeting different regions of the target DNA, can be more intricate compared to conventional PCR. This complexity may hinder the rapid development of new LAMP assays for emerging pathogens, limiting their broader application in diagnostic settings.

Multiplexing, or the ability to detect multiple pathogens in a single reaction, is another area where LAMP assays face challenges. While highly effective for single-target detection, LAMP may not be as suitable for multiplex assays as PCR. *Childress et al.* (2024) and *Zorkóczy* et al. (2023) discussed these limitations, which could restrict the assay's utility in creating comprehensive diagnostic panels needed for certain clinical scenarios.

Furthermore, LAMP assays have been observed to detect DNA from non-viable organisms, which can lead to false-positive results in infection diagnostics. *Lux et al.* (2023) emphasized the importance of careful result interpretation to distinguish between active infections and the presence of non-viable pathogens, highlighting critical consideration in clinical practice. Temperature control is another significant concern with LAMP assays. Maintaining precise isothermal conditions, typically around 60-65°C, is crucial for the success of LAMP reactions. *Nagamine et al.* (2002*)* and *Mansour* (2015) discussed the challenge of temperature regulation, particularly in field settings where maintaining consistent temperatures without specialized equipment can be difficult, potentially affecting assay reliability.

Lastly, while LAMP assays are highly sensitive for qualitative detection, they

generally provide limited quantitative data on pathogen load compared to quantitative PCR (qPCR). According to *Dhama et al.* (2014) while real-time LAMP (RT-LAMP) can offer some quantitative insights, its precision and accuracy in quantitative diagnostics do not match that of qPCR, which remains the gold standard for quantification in molecular diagnostics. While LAMP assays offer rapid, sensitive, and cost-effective pathogen detection, careful consideration of these limitations is essential to ensure their appropriate use and interpretation in clinical, field, and research settings. Addressing these challenges through ongoing methodological advancements and rigorous validation will further enhance the utility of LAMP assays in infectious disease diagnostics and surveillance.

5. Other isothermal amplification techniques

In the realm of molecular diagnostics, especially in settings requiring rapid and efficient testing, several isothermal amplification methods stand out, each with unique characteristics tailored to specific needs. Among these, LAMP has gained prominence due to its rapid processing time and high sensitivity, making it highly suitable for point-of-care applications. LAMP's ability to detect as low as one copy of the target per microliter and its use of multiple primers for enhanced specificity sets it apart from other techniques. This method is particularly advantageous in resource-limited settings because it does not require sophisticated laboratory equipment and allows for visual detection of results through turbidity or color change, facilitating quick diagnostics directly in the field (*Park*, 2022).

Comparatively, Recombinase Polymerase Amplification (RPA), usually completes reactions within 20 to 40 minutes, and operates effectively at lower temperatures (37-42°C). This makes RPA a strong contender for applications requiring quick turnaround without the need for thermal cycling *Zou et al.* (2020). The cost of the enzymes required for RPA and its sensitivity to sample impurities can limit its broader application, particularly in complex diagnostic scenarios where robustness and cost-efficiency are crucial (*Zou et al., 2020*).

Rolling Circle Amplification (RCA), another isothermal technique distinguishes itself by utilizing a simple mechanism that amplifies DNA through rolling circle replication. This method is particularly effective for analyzing small circular DNA molecules such as plasmids and viral genomes. Although its reaction time of about 90 minutes is slower than LAMP, RCA's ability to visualize amplification products with fluorescent dyes and its unique suitability for circular DNA templates provide significant benefits in virology and genetic research. However, RCA's slower reaction time and sometimes lower specificity compared to LAMP can be a drawback, especially in urgent diagnostic situations where speed and accuracy are paramount (*Li and Macdonald, 2015*).

By comparing these methods, it's evident that each has its strengths and limitations. LAMP, with its ability to provide rapid, reliable results with minimal equipment, proves to be highly advantageous in many scenarios. This makes it a preferred method, especially when immediate decision-making is essential in clinical or field diagnostics. The detailed discussion of the limitations of alternatives like RPA and RCA underscores the relative advantages of LAMP, ensuring a balanced perspective and justifying its recommendation for widespread diagnostic use.

6. Conclusions

LAMP assays and eDNA techniques represent powerful diagnostic tools with distinct advantages and applications. LAMP assays demonstrate exceptional sensitivity and specificity in pathogen detection, offering rapid results, simplicity, and cost-effectiveness, which are particularly beneficial in resource-limited environments. However, challenges such as potential contamination, complex primer design, and limited quantitative capability need to be addressed. Their adaptability to various environmental conditions enhances their versatility. Nevertheless, variability in detection efficiency due to environmental factors and the need for precise methodological protocols are areas for improvement. Future research should focus on addressing these limitations to enhance the diagnostic capabilities and broader applications of both LAMP and eDNA techniques and to promote their combined application. Collaboration between researchers, clinicians, and policymakers, along with investments in training and capacity-building initiatives, will be crucial for the effective implementation and advancement of these methods.

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