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Loop-Mediated Isothermal Amplification (LAMP)-Based Method for Rapid Mushroom Species Identification

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Supporting Information

ABSTRACT: Toxic mushroom species, such as the death cap (*Amanita phalloides*), are responsible for most mushroom poisonings. In the present work, novel loop-mediated isothermal amplification (LAMP) assays were used for the differentiation of even closely related edible and toxic mushroom species. The applicability of these methods was tested by cross-reaction studies and analysis of spiked mushroom samples (raw and fried material). Contaminations at the level of 2% (w/w) could be detected in different mushroom blends. Three detection methods were used: agarose gel analysis, fluorimetric real-time detection, and visual detection by lateral flow dipsticks (LFD). The LAMP assay combined with LFD detection allows the identification of *A. phalloides* in about 2 h (including DNA extraction) at a very low level of technical equipment (micropestle, water bath, and mobile centrifuge), which makes this technique perfectly suited for on-site applications.

KEYWORDS: *Loop-mediated isothermal amplification (LAMP), real-time detection, lateral flow dipsticks (LFD) visual detection, mushroom poisoning, Amanita phalloides*

INTRODUCTION

Loop-mediated isothermal amplification (LAMP) is a relatively new method, which allows amplification of DNA with high specificity and sensitivity under isothermal conditions. The method was originally described by Notomi et al.[1] and is mainly used for the detection of microorganisms and viruses; however, its use in the food sector is increasing rapidly.[2−7] The LAMP reaction requires a set of four primers. Two inner primers, the forward inner primer (FIP, composed of the hybridization sites F1c and F2) and the backward inner primer (BIP, composed of the hybridization sites B1c and B2), and two outer primers (F3/B3). The specially designed primers recognize six distinct sequences (F3c, F1c, F2c, B2c, B1c and B3c) of the target DNA (Figure 1A). Additionally, a DNA polymerase with strand displacement activity is mandatory, superseding thermal denaturation steps. In loop-LAMP assays, an acceleration of the reaction velocity is achieved by the addition of two extra loop primers.[8] The hybridization sites of these loop primers are located in the region between F2c and F1c (and B1c and B2c, respectively) (Figure 1A). During LAMP reaction, large amounts of amplification products are formed. Magnesium pyrophosphate is generated as a byproduct causing a turbidity, which can be detected by direct eye visual inspection or a turbidimeter.[9−12] Alternatively, LAMP products can be detected by either agarose gel electrophoresis (AGE) or visually using SYBR Green I, cationic polymers, hydroxynaphthol blue, or calcein.[2,11,13−18] In addition, the implementation of lateral flow dipsticks (LFD) enables a specific, rapid, and simple optical detection of LAMP products. LAMP−LFD methods are mainly described for the detection of shrimp and fish viral and bacterial infections;[19−21] only a few were applied to plant[22,23] and animal[22−24] diseases and the detection of genetically modified organisms.[7] Up to now, this technique was not used for mushroom species identification, especially in food analysis.

Fungi are the third largest group of eukaryotes after animals and plants. A total of 140 000 species of mushrooms have already been listed all over the world.[25] Picking and consumption of wild mushrooms is traditional in many countries.[25] Mushroom poisonings are almost always caused by ingestion of wild mushrooms that have been collected by non-specialists. It is difficult for untrained individuals to distinguish the different mushrooms because of hardly recognizable differences between poisonous and non-poisonous species. About 150 poisonous mushrooms are known in Europe.[26] Species producing amatoxin, like *Amanita* species, *Conocybe*, *Galerina*, and *Lepiota*, are responsible for the majority of mushroom poisoning cases. Some of these toxic mushrooms have no characteristic smell or taste; thus, they were eaten without misgiving. Initial symptoms of poisoning are delayed by 6 h or more.[27] Up to now, their identification is often mainly based on microscopic analyses of spores or cleaning residues or meal leftovers.[28] Beyond that, α-amanitin can be detected as a marker substance by enzyme-linked immunosorbent assays or radioimmunoassays.[28,29]

In Germany, 90% of the fatalities resulting from mushroom ingestion are associated with death cap (*Amanita phalloides*), whose toxicity has been explored since 1937.[30] The toxic substances are cyclopeptides, which can be divided into two groups of different toxicological compounds. The amatoxins, already mentioned above, are highly toxic and lethal within 8 ± 2 days.[30,31] The human LD_{50} for α-amanitin is about 0.1 mg/kg of body weight, and one mushroom cap can contain a fatal dose.[30] The phallotoxins are less toxic than the amatoxins but are also lethal.[32,33] Consequently, about 50 g of fresh weight,

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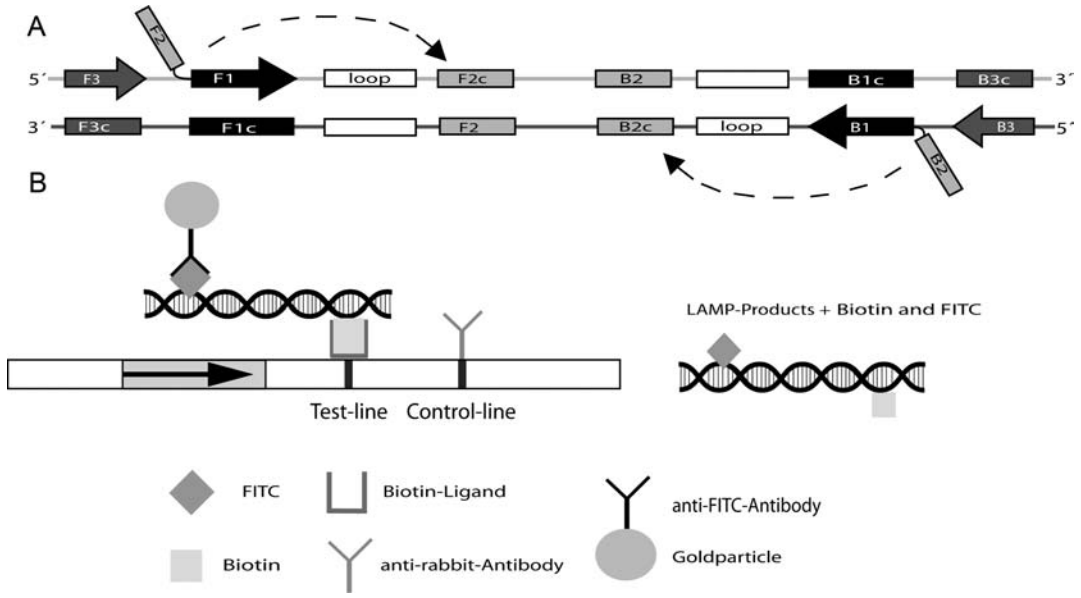

Figure 1. (A) Schematic diagram of the LAMP primer design, with FIP composed of F1 and F2, BIP composed of B1 and B2, two outer primers F3/B3, and the loop primer (c = complementary). (B) Schematic diagram of the LFD detection method.

i.e., one death cap, could be sufficient to kill a person. Cooking, freezing, or processing do not reduce the toxicity of these mushrooms.[34]

The aim of this study was to develop a specific DNA-based method for a rapid and easy detection of the death cap. In this study, frozen and cooked (fried) mushrooms were analyzed. The presented method was evaluated with respect to (i) DNA extraction, (ii) amplification, and (iii) detection. Criteria for the optimization were simplicity (i.e., minimizing the required technical equipment) and time-saving. This is the first described LAMP−LFD method for mushroom identification.

MATERIALS AND METHODS

Mushroom Samples. All mushroom species used in this study are listed in Table 1. Further information for all 19 samples used in this study is given in Table S1 of the Supporting Information. Mushroom samples were collected in forests of the north and northeast of Germany by mushroom experts of the "Pilz-Museum Neuheide" (Neuheide, Germany; 2 samples, designated as "PN" in Table S1 of the Supporting Information) and by Dr. W. Schultze (University of Hamburg; 11 samples, designated as "S" in Table S1 of the Supporting Information). The samples were stored at −20 °C. The wild mushrooms were authenticated by these mushroom experts with respect to morphological characteristics, sensory characteristics, and the habitat. The death cap sample was verified by sequencing the ITS 1 region and comparison to published sequences using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST). Additional reference material (cultivated mushroom) was bought in food stores (2 samples, designated as "CP" in Table S1 of the Supporting Information) or cultivated at the institute (Bio-Pilzhof & Edelpilzzucht Breck GbR, Malschwitz, Germany; 4 samples, designated as "C" in Table S1 of the Supporting Information). For preparation of fried samples, fresh mushrooms were heated in a frying pan with 1 mL of oil (sunflower oil) until a browning effect was observed. For the spiked samples, the mushrooms were homogenized in a blender and mixed. Different blends were prepared with death cap and edible mushrooms. For a first experimental setup, different amounts of death cap (20, 10, 1.50, and 0%) were added to a mixture of champignon mushroom (*Agaricus bisporus*), oyster mushroom (*Pleurotus ostreatus*), and king oyster mushroom (*Pleurotus eryngii*) (1:1:1). In a second experimental setup, the closely related European blusher (*Amanita rubescens*) was mixed with death cap (100, 50, 24, 9, 8, 2, 0.2, and 0%).

Table 1. Mushroom Samples Used in This Study[a]

family	species name	common name
Amanitaceae	***Amanita phalloides***	**death cap**[b]
	Amanita muscaria	**fly agaric**[b]
	Amanita pantherina	**panther cap**[b]
	Amanita citrina	**false death cap**[c]
	Amanita rubescens	**European blusher**[d]
Agaricaceae	*Agaricus bisporus*	champignon mushroom[d]
Pleurotaceae	*Pleurotus ostreatus*	oyster mushroom[d]
	Pleurotus eryngii	king oyster mushroom[d]
	Pleurotus citrinopileatus	golden oyster mushroom[d,e]
Marasmiaceae	*Lentinula edodes*	shiitake mushroom[d]
Boletaceae	***Xerocomus badius***	**bay bolete**[d,e]
	Xerocomus chrysenteron	**red cracking bolete**[d,e]
	Leccinum scabrum	**birch bolete**[d,e]
	Boletus edulis	**king bolete**[d,e]
Cantharellaceae	*Cantharellus cibarius*	golden chanterelle[d,e]
Strophariaceae	***Kuehneromyces mutabilis***	**sheathed woodtuft**[d,e]
Tricholomataceae	***Lepista nuda***	**wood blewit**[d,e]
	Calocybe gambosa	**St. George's mushroom**[d,e]
Russulaceae	***Lactarius deterrimus***	**false saffron milk-cap**[d,e]

[a]Wild mushrooms collected in the forest are in bold font. [b]Poisonous mushroom. [c]Poisonous as a raw mushroom. [d]Edible mushroom. [e]Used for specificity tests.

DNA Extraction. Mushrooms (fruit body) of about 150 mg (fresh weight or fried sample, respectively) were placed in a 2 mL microfuge tube, adding 200 μL of chloroform. The samples were crushed either mechanically by a TissueLyser (Qiagen, Hilden, Germany; 3 min, 30 Hz) using two sterile stainless-steel beads ($^1/_8$ in.) or manually with a spatula tip of quartz sand (~100 mg) and micropestle until a homogeneous mixture was obtained. After approximately 3 min of grinding time, most samples were already completely homogenized. After the addition of 400 μL of cetyltrimethylammonium bromide (CTAB) solution [0.1 M tris(hydroxymethyl)aminomethane (Tris)/HCl, 55 mM CTAB, 1.4 M NaCl, and 20 mM ethylenediaminetetraacetic acid (EDTA) at pH 8.0], the respective homogenization procedure was repeated. A total of 200 μL of guanidinium thiocyanate

Table 2. Primer Sequences Used in This Study (LAMP Primer and PCR Primer)

name	sequence (5′ → 3′)
F3	CCTTGCGCTCCTTGGCATT
B3	CAAGTTGTCCCCATCCATATAA
FIP	biotin-TTTTTATTTGAAACAGCCTGCAACCCCC*taat*GAGCATGCCTGTTTGAGTGTC
BIP	GAATGTATTAGTGGAGAAAAGCCATTGAACT*tata*TTATACAGACAGTCACAGTTAGAC
Loop-Rw	GCCAGGAGCAATATCACTTC
Loop-Fw (probe)	FITC-AAAAGCAGACAGGTCTTGAGA
Uni5.8s-FW	AACGGATCTCTTGGCTCTCG
Uni5.8s-RW	TGACACTCAAACAGGCATGC

(5 M) was added; the solution was mixed and incubated at room temperature for 5 min. After centrifugation (2000*g* for 2 min), the supernatant was mixed with 300 μL of isopropanol and incubated for 5 min on ice. The solution was placed on an EconoSpin All-in-One Mini Spin Column (Epoch Biolabs, Missouri City, TX) and centrifuged at 2000*g* for 2 min. The flow through was discarded, and the column was washed twice with 500 μL of 70% ethanol by centrifugation at 2000*g* for 2 min. For a complete removal of the ethanol, the column was centrifuged at 2000*g* for 2 min. Finally, the spin column was transferred to a new 1.5 mL microfuge tube, and DNA was eluted with 50 μL of warm water.[35] DNA concentration was adjusted to 1−10 ng/μL and stored at −20 °C until use. For on-site applications, it is possible to operate the centrifuge with a mobile battery.

The isolated DNA was analyzed and evaluated in terms of purity and concentration. The purity of DNA extracts was evaluated by the 260/280 nm extinction ratio. Concentrations of DNA extracts were measured fluorometrically using a SYBR Green I assay (Invitrogen, Eugene, OR).[36] To verify the amplifiability of the isolated DNA, a polymerase chain reaction (PCR) was carried out with universal primers, which anneal at the conserved region of 5.8S ribosomal deoxyribonucleic acid (rDNA) (Table 2).

PCR Conditions. PCR was performed using a Biometra thermal cycler (T3000, Biometra, Göttingen, Germany). After an initial denaturation at 95 °C for 3 min, 35 cycles at 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s were carried out. For a terminal elongation, the reaction batches were incubated for 5 min at 72 °C.

Reaction mixtures for qualitative PCR contained *Dream*Taq-Polymerase (0.5 unit/25 μL, Fermentas GmbH, Sankt Leon-Rot, Germany) together with the supplied *Dream*-Taq buffer, deoxyribonucleotide triphosphates (dNTPs, 200 μM each, Bioline GmbH, Luckenwalde, Germany), 0.5 μM of each primer (Invitrogen, Life Technologies GmbH, Darmstadt, Germany), and 2.5 μL of the DNA solution in a total volume of 25 μL.

Design of LAMP Oligonucleotides. A total set of six LAMP primers were designed for the specific detection of death cap based on the published sequence of the highly variable internal transcribed spacer region (NCBI accession number GQ221839.1) according to Notomi et al.[1] The primer set consisted of two outer (F3 and B3), two inner (FIP and BIP), and two loop primers (Loop-Fw and Loop-Rw). The inner primers cover two distinct sequences of the target (F1c/B1c and F2c/B2c) and are connected with a link of 4 base pairs. Sequences of the LAMP primers are given in Table 2. A schematic overview on the hybridization sites are shown in Figure 1A.

For the LFD detection, primer FIP was 5′-biotin-labeled and the Loop-Fw primer was 5′-FITC-labeled; the latter was used as a DNA probe after the LAMP reaction. Unlabeled primers were synthesized by Invitrogen (Life Technologies GmbH, Darmstadt, Germany), and the labeled primer (biotin) and probe (FITC) were synthesized by Metabion (international AG, Planegg-Martinsried, Germany).

LAMP Reaction. The real-time LAMP reaction was carried out in two steps in a total volume of 20 μL.

LAMP Mixture 1. A total of 8 μL containing FIP and BIP (4.5 μM each), the outer primers F3 and B3 (0.5 μM each), 17.5% dimethyl sulfoxide (DMSO), 1 μL of ThermoPol buffer (New England Biolabs, Frankfurt, Germany), and SYBR Green I (1:40 000 dilution of a 10 000-fold concentrate in DMSO delivered by Invitrogen, Life Technologies GmbH, Darmstadt, Germany) were mixed with 4 μL of DNA solution (set to 1−10 ng/μL). In experiments with loop primers, one or two of these primers were added with a final concentration of 2 μM. After incubation at 95 °C for 5 min, this LAMP mixture 1 was chilled on ice.

LAMP Mixture 2. This mixture contained 1 μL of ThermoPol buffer (New England Biolabs, Frankfurt, Germany), 1 mM of each dNTP (Bioline, Luckenwalde, Germany), and 10 units *Bst*-DNA-polymerase (Epicenter Biotechnologies, Madison, WI, or New England Biolabs, Frankfurt, Germany) in a total volume of 8 μL.

LAMP mixtures 1 and 2 (total volume of 20 μL) were mixed and incubated at 62 °C for 80 min (45 min, if the loop primer was used). Finally, the polymerase was inactivated at 80 °C for 10 min.

For no template controls (NTCs), LAMP mixture 1 contained 4 μL of H_2O instead of DNA template.

AGE. PCR or LAMP reaction batches (10 μL) were analyzed on 2% agarose gels (120 V) using TAE buffer (40 mM Tris/acetate and 2 mM EDTA at pH 8.2), followed by ethidium bromide (0.001%) staining. The results were documented under ultraviolet (UV) light (Biostep, Felix 1040, Biostep GmbH, Jahnsdorf, Germany).

Real-Time LAMP. Real-time LAMP or loop-LAMP amplifications were performed with LAMP mixtures 1 and 2 as described above using an iQ5 real-time PCR detection system (Bio-Rad, Munich, Germany). The data generated with SYBR Green I were plotted as the relative fluorescence signal versus time (each cycle was set to 1 min at a constant temperature of 62 °C).

The threshold cycle (C_t) used for real-time PCR assays was converted into threshold time (T_t), i.e., the time (minutes) required for the fluorescence signal to cross an arbitrary threshold fluorescence. Using a real-time detection system, the LAMP assay could be observed in consideration of a correlation between the DNA concentration and required time for a positive result.[37,38]

LFD Detection. The detection with LFD was carried out using commercially available dipsticks fabricated by Milenia Biotec GmbH (Gießen, Germany, Milenia HybriDetect). These sticks detect biotin-labeled DNA, which has been hybridized with a FITC-labeled DNA probe in a test zone containing an immobilized biotin ligand. Additionally, a control zone at the top of the stick contains immobilized anti-rabbit antibodies (see Figure 1B).

First, LAMP experiments were performed as described above, using a biotinylated FIP primer. Subsequently, the FITC-labeled DNA probe (20 pmol) was added to the LAMP products for hybridization at 62 °C for 5 min. A total of 8 μL of this hybridized product was mixed with 100 μL of the HybriDetect assay buffer (Tris-buffered saline) containing gold-labeled anti-FITC antibodies (rabbit), resulting in a LAMP product (biotin-labeled)/DNA probe (FITC-labeled)/antibody complex. Finally, the LFD was dipped in this solution[24,39,40] until the control band was visible at the control zone.

RESULTS AND DISCUSSION

The aim of this study was to develop a rapid and simple system for the identification of the poisonous mushroom death cap, which can be performed on-site and can be adapted to other issues of the food sector. The first step was the development of a rapid and efficient DNA extraction method.

DNA Extraction. First of all, a method to disrupt the fungal cell wall had to be developed. The disruption of the samples is

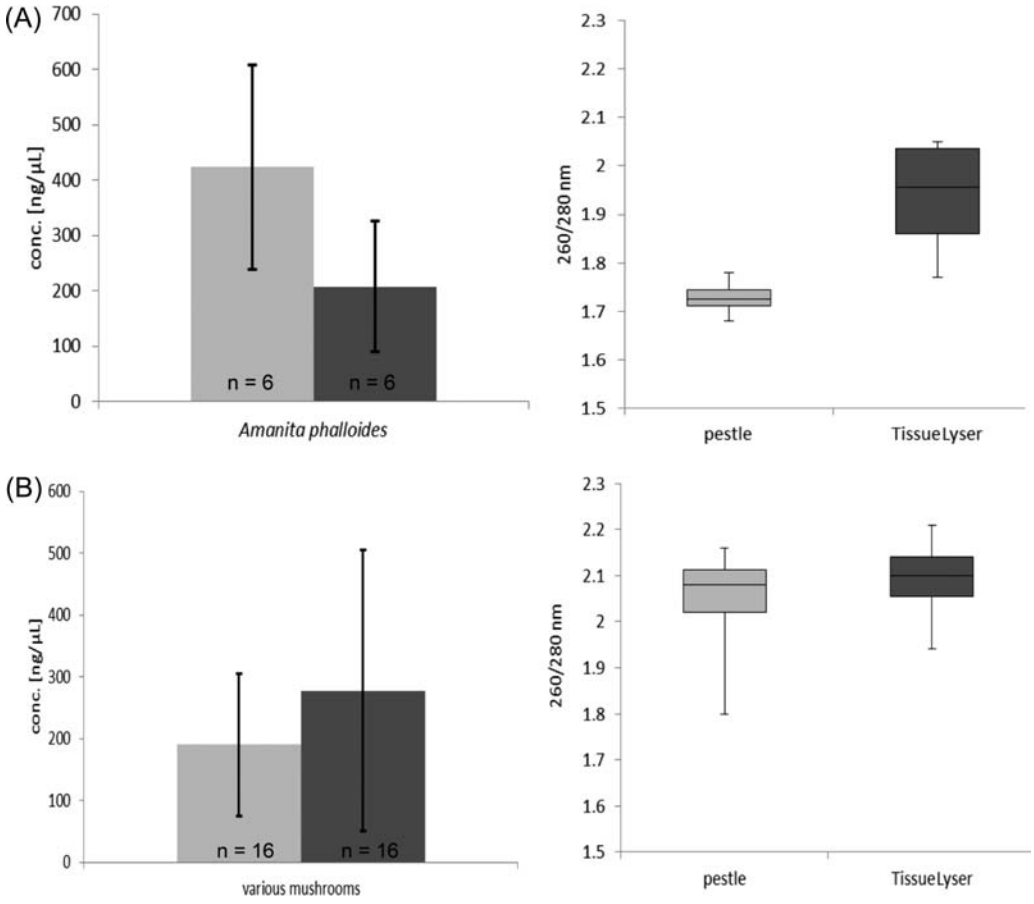

Figure 2. DNA yield (average with standard deviation, left) and DNA purity (box and whiskers blots, right) from isolated DNA from fresh or fried death cap (A) and eight other mushrooms (isolated in duplicates; fresh) listed in Table 1 (B). DNA isolation was performed using either the TissueLyser (dark gray) or the pestle (light gray) method.

mandatory for the isolation of the DNA. The structure of the fungal cell wall is highly complex compared to mammalian cell membranes and bacterial cell walls. Enzymatic processes, such as proteinase K digestions, which are often described in refs 41−43, are not applicable for a rapid method because of their long incubation times. Physical disruption methods (mortar and pestle grinding or TissueLyser) are frequently used. According to Karakousis et al., the efficiency of lysis using mortar and pestle is very high.[44] In agreement with the results for mechanical lysis, the TissueLyser offers high-throughput sample disruption in the laboratory. For the on-site method, quartz sand and pestles were used. The whole on-site extraction takes about 45 min for 6−8 samples. Both methods resulted in high yields and sufficient DNA quality from death cap (Figure 2A). The results were not influenced by the condition of the starting material. Even with fried fruit bodies and the use of the TissueLyser, high DNA amounts of about 100 ng/μL on average (45−164 ng/μL) were obtained. Notably, DNA isolation by quartz sand and pestle resulted in higher DNA yields than using the TissueLyser. As expected, using different mushroom matrices, DNA yield and purity varied in a much higher range (Figure 2B). Some DNA solutions were slightly stained, but the subsequent dilution step to adjust the required DNA concentration of 1−10 ng/μL also diluted the co-isolated staining compounds to an acceptable level. It is important to state that every isolated DNA was amplifiable in PCR after a dilution step.

LAMP Conditions. LAMP was carried out using 40 ng of death cap DNA/assay as the template to determine the optimal temperature and reaction time and to evaluate the use of loop primers. LAMP product formation could be detected at a temperature range of 60−64 °C. The electrophoresis pattern of these experiments showed typically ladder-like banding, with little difference between conditions. Consequently, 62 °C was considered to be the optimal reaction temperature for LAMP assays.

Using a real-time SYBR Green I detection system, the kinetic of a LAMP assay could be monitored, enabling the determination of the minimum time for the detection of a positive reaction. As seen in Figure 3A, using 40 ng of DNA/assay, the LAMP reaction is finished after about 1 h. As known from real-time PCR experiments, a linear relationship between the DNA concentration and T_t could also observed for the LAMP assays. Furthermore, so-called "background amplifications" were observed. These unspecific amplifications are known to occur in NTCs after longer incubation times.[45] However, it should be noted that, in real-time LAMP assays, the background amplifications could clearly be differentiated from the specific reactions because of their higher T_t values. In this study, it could be observed that background amplification showed a strong dependency upon primer design, primer concentration, and the number of primers that were used (i.e., additional loop primer). Hence, for the loop-LAMP assay with LFD detection, only one loop primer was used to suppress background amplification and the formation of primer dimers,

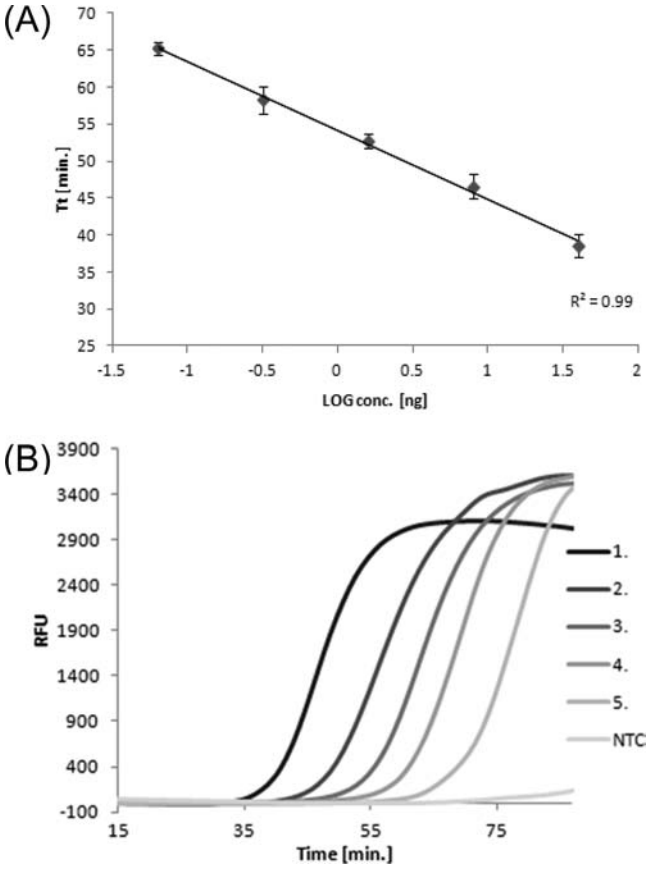

Figure 3. (A) Standard curve calculated from panel B. Every DNA concentration was measured 4-fold. Standard deviations are shown. (B) Real-time LAMP using a dilution series of death cap DNA: 1, 40 ng; 2, 8 ng; 3, 1.6 ng; 4, 0.32 ng; 5, 0.064 ng; and NTC, no template control.

which also could lead to false-positive results with the real-time SYBR Green I detection system.

Specific LAMP Assay. To test the specificity of the LAMP primers, the system was tested with different fungal DNA matrices, including DNA from related *Amanita* species, such as *A. muscaria*, *A. citrina*, *A. pantherina*, and *A. rubescens*, and DNA from the most popular edible mushrooms, such as *Agaricus bisporus*, *Lentinula edodes*, *Pleurotus ostreatus*, and *Pleurotus eryngii*. Additional mushrooms that were tested are listed in Table 1. No cross-reaction leading to false positives was detected in these cases. In Figure 4, some results of the specificity tests are exemplarily shown. With all three detection systems, a positive LAMP reaction could be detected exclusively with DNA from death cap. To ensure that fried death caps could also be detected, a real-time LAMP assay was performed with DNA from raw and fried death caps. As seen in Figure 5, the T_t values varied in a LAMP typical range. However, it should be noted that the limited numbers of species of *Amanita* in the collection of the institute did not represent all species of *Amanita*. Hence, the specificity of the method is only assured for the species under study under the given conditions. Nevertheless, the LAMP method could be expanded relatively easily to include more species.

Sensitivity. To analyze the sensitivity of this system and to evaluate the linear working range, real-time LAMP experiments with death cap DNA dilutions ranging from 40 to 0.064 ng (1:5 dilution series) were performed (Figure 3). The standard curve based on these dilution series showed a linear relationship between the log of the quantity of initial template DNA and T_t.

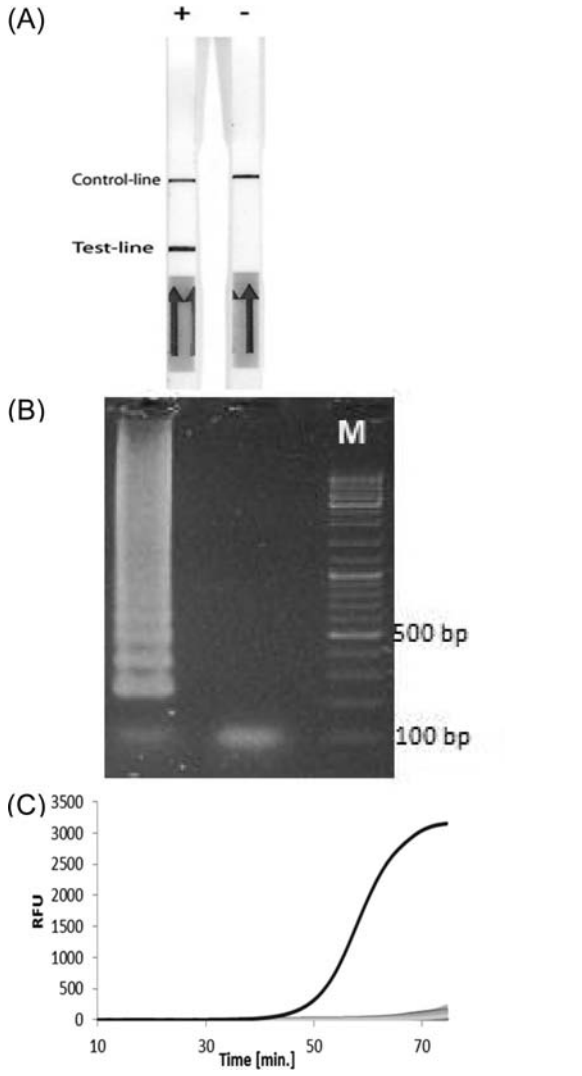

Figure 4. Specificity tests of the death-cap-specific LAMP exemplarily shown are positive and negative results and different systems for detection. (A) LFD: +, positive death cap; −, negative for different mushrooms. (B) AGE: see panel A for description. M, DNA ladder of 100−10 000 bp. (C) Real-time system: black line, death cap; gray lines, different mushrooms.

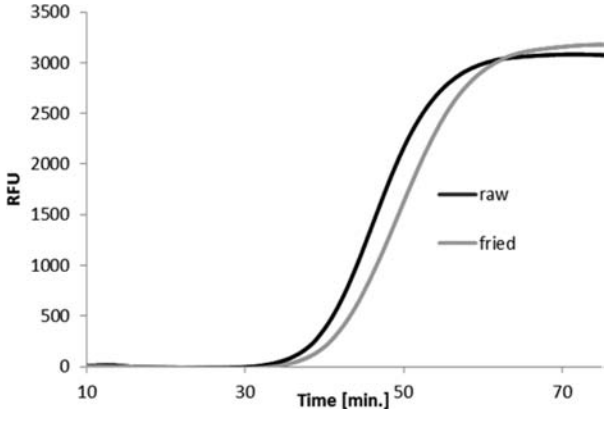

Figure 5. Real-time detection of LAMP assays using DNA isolated from fresh (black) and fried (gray) death caps.

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The coefficient of determination (R^2) of the linear regression was 0.99 (Figure 3B).

In Figure 6, the results of real-time LAMP measurements of spiked samples are shown. For the experiments, a mixture of

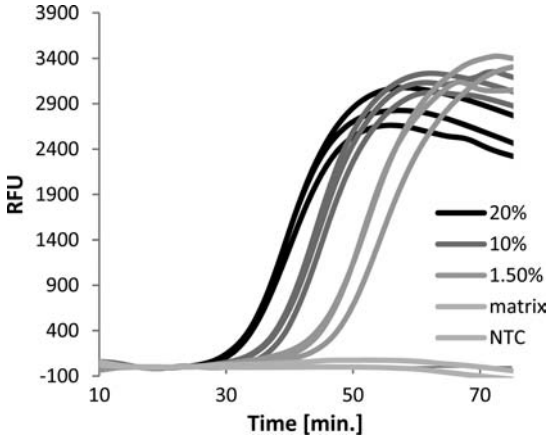

Figure 6. Sensitivity tests of the death-cap-specific LAMP using DNA from an edible mushroom mixture spiked with different amounts of death cap (matrix: 20, 10, 1.50, and 0%) of a real-time system. NTC = no template control.

edible, i.e., nontoxic, mushrooms (cf. Materials and Methods) was spiked with variable amounts of death cap and the DNA was isolated. All three detection methods were used, and death cap was detectable up to 1.5%. The addition of one loop primer accelerated the reaction to approximately 2 times.

To verify the specificity and sensitivity, death cap was mixed with the closely related edible mushroom European blusher (*A. rubescens*) in different amounts. The LAMP reaction was performed using one loop primer. Using the LFD detection system, a faint band was still observed for the 0.2% mixture at which concentration an amplification product was hardly visible by AGE detection (Table 3). These results confirm the observations by Kiatpathomchai et al., who described a higher sensitivity for LFD compared to AGE.[39]

Table 3. Results of the Sensitivity and Specificity Tests of the Death-Cap-Specific Loop-LAMP Using DNA from a Mixture of Death Cap with the Closely Related Edible Mushroom European Blusher (*A. rubescens*) by LFD Detection and AGE[a]

death cap (%)	100	50	24	9	8	2	0.2	0	NTC
LFD detection	+	+	+	+	+	+	+	–	–
AGE	+	+	+	+	+	+	(+)	–	–

[a] +, positive result; (+), hardly visible; –, negative result; and NTC, no template control.

With the developed LAMP methods, the detection of the toxic mushroom, which accounts for the majority of fatal mushroom poisonings,[46] is possible in different mushroom blends. According to the Bundesinstitut für Risikobewertung (BfR), one-time consumption of about 225 g of fresh mushrooms is possible for an adult. Assuming that one death cap has an average weight of 50 g and that the lethal doses of toxins can be present in such a single mushroom, the detection of one death cap in 225 g (22%) is demanded.[30,47] Using the present LAMP method even $^1/_{10}$ of the lethal dose can easily be detected. It should be noted that the detectable value may slightly vary because of different moisture levels of different mushrooms.

In conclusion, the present LAMP method proved to be a rapid and accurate method of identification for the toxic death cap for raw and fried material. Even in different mushroom blends, 2% (w/w) of contamination can be detected. This is of particular importance for mushroom ingestion cases, because fast identification might help health care professionals.

The use of a LFD system for the detection of LAMP amplification products had the advantage (i) to decrease the overall experimental time and (ii) to simplify the experimental setup. With LAMP–LFD, the identification of death cap could be completed in 2 h only using a heating system and a battery-operated centrifuge. This method can also easily be adapted to other target genes and is effective for the screening of many samples.

■ ASSOCIATED CONTENT

Supporting Information

Designation of all 19 mushroom samples used in this study (Table S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

AGE, agarose gel electrophoresis; CTAB, cetyltrimethylammonium bromide; F3 and B3, forward and backward outer primers; FIP and BIP, forward and backward inner primers; LAMP, loop-mediated isothermal amplification; LFD, lateral flow dipsticks; NCBI, National Center for Biotechnology Information; NTC, no template control; PCR, polymerase chain reaction; rDNA, ribosomal deoxyribonucleic acid; Tris, tris(hydroxymethyl)aminomethane; T_t, threshold time

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