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An Application of Loop Isothermal Amplification to Detect African Swine Fever Virus

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Abstract

Loop isothermal amplification (LAMP) is an alternative to PCR that reduces the time of analysis and does not require the use of expensive equipment. Here, we describe our real-time LAMP technique with hybridization fluorescence detection for the detection of ASF virus in biological material from animals. The detection limit of the technique is $2*10^3$ copies/ml, which is comparable to the sensitivity of PCR. The high specificity of the method developed by LAMP was shown, and no false positive results were observed when testing other viruses and microorganisms, as well as biological samples that did not contain DNA of the ASF virus. In comparative tests on blood plasma samples (n=33) and a suspension of pathological material of the tonsils, spleen, lungs (n=30), the results of PCR and LAMP coincided almost completely, with the exception of one LAMP false-negative sample with a low viral load (PCR Ct ~ 38).

Disciplinary: Veterinary.

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1 Introduction

African swine fever (ASF) is a highly contagious viral disease of swine, characterized by fever, cyanosis of the skin and extensive hemorrhages in the internal organs. Refers to especially dangerous contagious animal diseases that have the ability to be dangerous and rapid spread.

The introduction of the PCR method in the diagnosis of ASF has significantly increased the efficiency of the timely interpretation of this etiology, which is associated with its high sensitivity and specificity. Despite good analytical and diagnostic characteristics, the PCR method requires expensive equipment, and the study takes a lot of time. Taking into account the stage of nucleic acid isolation, the time to obtain the result of a PCR study is about 4 hours [13].

Methods of isothermal amplification, which appeared in the 21st century, are successfully used to detect a wide range of pathogens of infectious diseases. Their advantage is to reduce the amplification time from several hours to 15-30 minutes while maintaining high sensitivity and specificity. Another important advantage of isothermal methods is the possibility of their application in conditions of limited resources without the use of complex equipment, since the isothermal reaction can proceed at a constant temperature in a thermostat, and the results can be detected visually, for example, due to the turbidity of the solution due to the appearance of a precipitate of magnesium pyrophosphate. [1] or discoloration of pH-sensitive dyes [2].

Due to the current lack of effective vaccines to prevent ASF, early identification and elimination of affected animals is an important means of controlling and eradicating ASF. In recent years, various methods for express and laboratory diagnosis of ASF have been developed, which have good diagnostic performance and allow timely measures to be taken at early stages to curb the development of epidemics [3, 4].

The PCR method is the gold standard of molecular diagnostics, however, the development of modern fast and simple molecular techniques based on isothermal DNA amplification, while maintaining the high accuracy and sensitivity characteristic of PCR-based studies, can make ASF diagnostics faster and more affordable for use [5, 6].

The isothermal amplification method provides for minimal requirements for testing (the reaction is carried out at one temperature regime), the testing time, including the nucleic acid extraction stage, takes about an hour, by reducing the time required for amplification, which brings nucleic acid amplification methods closer to the express test format. [12].

However, the use of these express tests in well-equipped laboratories is not justified, due to the inability to control the loss of nucleic material during their extraction, as well as the lack of control over the efficiency of purification of nucleic material from substances that inhibit the amplification reaction.

In well-equipped laboratories, LAMP with detection based on hybridization fluorescent probes can be used as a fast alternative to PCR. This approach to the detection of LAMP results, by analogy with multiplex PCR, makes it possible to multiplex reactions in one tube using different fluorescent labels to detect the DNA of different targets [14].

In this work, a LAMP with hybridization-fluorescence detection was developed to detect ASF virus DNA in the presence of an internal control sample, and the diagnostic characteristics of this technique were compared with PCR.

2 Materials and Methods

2.1 Clinical Samples and Their Sample Preparation

Samples of biological material - blood plasma (n=42) and tissue suspension (tonsils, spleen, lungs) (n=21) from animals were obtained from farms in the Moscow, Vladimir, Kaluga regions and Primorsky Krai (Russia).

Tissue material (volume 200-300 µl) was homogenized using porcelain mortars, and a ~10% (v/v) suspension was prepared in phosphate-buffered saline (VWR International, LLC, USA). The suspension was allowed to stand for 2-3 min and 100 µl of the upper phase of the suspension was used for DNA extraction. The clarified extract was stored at -20 °C. until testing.

DNA extraction. The NK-set-OD kit (OD-Test, Russia) was used to isolate the nucleic material from the clarified fecal extract. Extraction is based on lysis using a solution of guanidine isothiocyanate followed by precipitation of the nucleic acids with alcohol and dissolution in TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0).

During DNA extraction, exogenous DNA of the internal control sample (ICS), which is the DNA of the bacteriophage lambda, was added to each sample (FGBU NRC Kurchatov Institute, GosNIIgenetika, Russia).

PCR. The presence of ASF virus genetic material in the biological material was determined using two kits from different manufacturers: PCR-ASF-Factor (VetFactor, Russia) and ODT-ASF (OD-Test, Russia). PCR was performed on CFX96 RealTime PCR machines (Bio-Rad, USA) and RotorGene Q (Qiagen, Germany).

2.2 Selection of Primers for LAMP

Table 1: LAMP primers for the detection of ASF virus DNA

primer name	sequence (5′-3′)	length
ASF_F3	ATTACGTCTTATGTCCAGA	19
ASF_B3	AGACTGGATATAAGCACTTG	20
ASF-FL	TCACAATATCCAAACAGCAGGT	21
ASF-BL	TAAGAATAGGTTTGCTYTGGTG	20
ASF-FIP	ACCAACCCGAAATTCCTTTCACtttTTGCGTCCGTAATAGRGTRATA	48
ASF -BIP	GTTCGCTCGTATCATTTTCATttttAGGTATCGGTGGAGGGAACY	46
IC_F3	aaacgcaacgaggctcta	18
IC_B3	GGATTTGTTCAGAACGCTC	19
IC-FL	ACAGCTTCCGCTGTCTTCTCA	21
IC-BL	gttgacgacgacatggctc	20
IC-FIP	CCTGCTGATCTGCGACTTAtttcgagagtgcgttgcttaac	41
IC -BIP	ggattccaaagttctcaatgcttttGAGAATCGCAGCAACTTGT	44
ASF- Probe	R6G Probe – TCACAATATCCAAACAGCAGGT **	
IC-Probe	FAM Probe - ACAGCTTCCGCTGTCTTCTCA **	
Q	Quencher -BHQ1**	

* Degenerate nucleotide designations: Y = C or T, R = A or G.

** Nucleotide sequences similar to the nucleotide sequence of *Probe* and its complementary *Quencher* have been published previously [9]..

For multiple alignments of the known sequences of the ASF virus, the Mega X program and the Clustal W algorithm were used [7]. LAMP primers were selected using the Primer Explorer V software (Fujitsu, Japan) and the NCBI BLAST homologous sequence search resource [8]. Polymorphic positions among the ASF virus sequences in the primer region were accounted for by degeneracy. Primers for amplification of the internal control sample, bacteriophage lambda DNA, were selected in the region of the right arm of the bacteriophage genome, Early Right Operon, (NC_001416, 38375–38639 bp).

The sequences of LAMP primers for detecting ASF virus DNA and lambda bacteriophage DNA are shown in Table 1. Primers and probes were synthesized and purified using high-performance liquid chromatography, Evrogen, Russia.

2.3 Real-time LAMP With Probe

Loop modified probe primers labeled at the 5' end with a fluorescent label in a duplex with the Quencher-BHQ1 oligonucleotide complementary to the part of the loop primer probe and labeled at the 3' end with a fluorescence quencher were used for real-time detection of LAMP [8] (Table 1).

The reaction mixture contained the following components: primers for amplification of ASF virus DNA and lambda bacteriophage DNA (Table 1) at the following concentrations ASF_FIP, ASF_BIP, IC_FIP, IC_BIP - 2 μM each, ASF_F3, ASF_B3, IC_F3, IC_B3 - 0.5 μM each, ASF_FL, IC_FL - 0.08 μM, ASF_BL, IC_BL - 1.6 μM, primer probes ASF-Probe, IC-Probe 0.08 μM each, quencher Q - 0.20 μM, 5 μl 5x LAMP buffer (OD-Test, Russia), 8 units.a. Bst polymerase (JSC Genterra, Russia), 1.4 mM of each dNTP (Biosan, Russia), 10 μl of DNA probe, sterile water to a final volume of 25 μl.

Positive and negative amplification controls were included in each experiment.

To detect LAMP results, a thermal cycler CFX96 RealTime PCR machine (Bio-Rad, USA) was used. Amplification program: 65 °C, fluorescence detection every 30 sec, 70 rounds. Two rounds of amplification (2 Ct) corresponded in duration to about 1 minute.

2.4 Obtaining a Positive Control Sample

The reaction mixture for obtaining a PCR product of the p32 gene fragment of the ASF virus contained the following components: 10 μl of a DNA sample isolated from a biological material containing the ASF virus; primers ASF_F3, ASF_B3 - 0.4 μM each; dNTP (Biosan, Russia) 0.2 mM; TaqF polymerase (JSC Genterra, Russia) 5 u.a., Tris-HCl-buffer (pH 8.3) with a concentration of tris (hydroxymethyl)-aminomethane 70 mM (Sigma-Aldrich, USA), magnesium chloride - 4 mM (Sigma-Aldrich, USA), potassium chloride - 80 mM (Sigma-Aldrich, USA), enzyme stabilizer - 0.2 mg/ml (JSC Genterra, Russia), volume - up to 25 μl. Thermal cycling program: 95°C - 15 min; the following stages were repeated for 40 cycles - 95°C - 15s, 60°C - 30s, 72°C - 15s. Amplification was carried out on CFX96 RealTime PCR machines (Bio-Rad, USA).

The pAL2-TA plasmid vector (Evrogen, Russia) was used to clone the PCR product at the TA sticky ends. The insertion sequence of the recombinant plasmid pPKO_ASF was confirmed by

sequencing.

The pPKO_ASF plasmid DNA concentration was assessed using digital PCR (dPCR) on a Quant Studio 3D system (Thermo FS) using a QuantStudio™ Digital Digital PCR Chip Kit v2 and external LAMP primers ASF_F3, ASF_B3, 0.25 mM each. Thermal cycling program: 95°C - 5 min; 40 cycles - 94°C - 30s, 60°C - 60s, then 4°C - 5 min, 90°C - 5 min.

2.5 Real-time LAMP Sensitivity and Specificity Determination with Probe

The sensitivity of LAMP methods was determined using dilutions of DNA pPKO_ASF with concentrations of $4*10^2$ copies/ml, $2*10^3$ copies/ml, 104 copies/ml, tested in seven repetitions each. The detection limit was considered to be the minimum concentration of PCO, reproducibly detected in all repeats.

To test the specificity of the developed methods, we used strains of viruses and microorganisms from the collection of the FGBU VGNKI - Classical swine fever virus, Porcine circovirus 2, Porcine epidemic diarrhea virus, Porcine parvovirus, Porcine reproductive and respiratory syndrome virus, Rotavirus A, Suid alphaherpesvirus 1, Transmissible gastroenteritis virus, Brucella suis; Campylobacter jejuni; Chlamydia suis; Escherichia coli; Haemophilus parasuis; Listeria monocytogenes; Pasterella multocida; Salmonella Dublin; Shigella flexneri; Staphylococcus aureus; Yersinia pseudotuberculosis. RNA/DNA was isolated from the strains using the NK-set-OD reagent kit (OD-Test, Russia).

2.6 Statistical Data Analysis

The diagnostic performance of LAMP was calculated using the MEDCALC Internet resource [10] by entering the values of true (TP) and false (FP) positive and true (TN) and false (FN) negative results.

3 Result and Discussion

3.1 Real-time LAMP Design with Probe

A fragment of the p32 gene was used as a target for LAMP since this region of the genome is quite conservative among the sequences of African swine fever virus (NCBI taxon: txid10497) and has significant differences from the sequences of other viruses and microorganisms. Six LAMP primers spanning 8 regions of the p32 gene and a primer-probe for LAMP with fluorescence detection were selected. BLAST comparison with the known sequences of the ASF virus revealed single polymorphisms, the most significant of which at the 3' ends of the primers were taken into account by the degeneracy of their sequences. BLAST search for homologous sequences in NCBI databases showed no significant identity of the selected primers with sequences of other taxa.

For real-time detection of LAMP, we used a modified loop primer probe with an additional Probe nucleotide sequence and labeled it with a fluorescent label at the 5' end of R6G [9]. The modified loop primer probe is hybridized to a small complementary Q-BHQ1 oligonucleotide labeled at the 3' end with a fluorescence quencher. In the formed duplex, the R6G fluorophore and

the BHQ1 quencher are in close proximity; during LAMP, the polymerase displaces the quencher from the 5' end of the loop primer, which leads to the accumulation of a fluorescent signal in the reaction mixture [9].

For real-time amplification of the internal control sample, LAMP primers IC_F3, IC_B3, IC-FL, IC-BL, IC-FIP, IC-BIP in the region of the right arm of the genome of the bacteriophage Early Right Operon (Table 1) and a modified loop primer probe with additional nucleotide sequence Probe and labeled at the 5' end of FAM with a fluorescent label, hybridized with quencher oligonucleotide Q-BHQ1 [9].

3.2 Determination of the Analytical Sensitivity and Specificity of LAMP in Real Time with a Probe

To determine the analytical sensitivity of LAMP, solutions of serial dilutions of plasmid DNA pPKO_ASF containing a fragment of the p72 gene of the ASF virus were used at concentrations of $4*10^2$ copies/ml, $2*10^3$ copies/ml, 10^4 copies/ml.

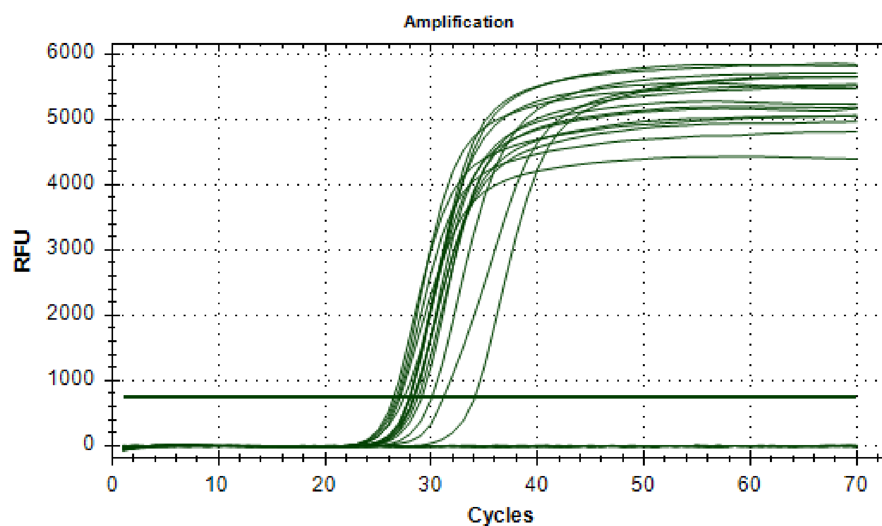

Figure 1: Analytical sensitivity of LAMP with fluorescent probe. Dilutions of pPKO_ASF with concentrations of 2*10^4 copies/ml, 2*10^3 copies/ml, 2*10^2 copies/ml were used, which corresponds to the addition of 200, 20 and 2 copies of the target DNA to the reaction mixture, respectively. NC - negative amplification control, Sus scrofa DNA. The reaction time is 35 minutes.

The LAMP method with a probe makes it possible to detect ASF virus DNA at a concentration in the test sample from 2*10^3 GE/ml, which corresponds to 20 copies of DNA per reaction, showing positive amplification in all seven repeats for this concentration. Thus, the LAMP method shows a high analytical sensitivity comparable to PCR methods [13].

The specificity of LAMP against the ASF virus was confirmed on the NK of various strains of viruses and microorganisms (Table 2). To determine the specificity of the LAMP methods, the reaction time was increased to 60 min. No cross-reaction with other organisms has been identified.

Determination of diagnostic sensitivity and specificity of LAMP in real-time with a probe.

To test the diagnostic sensitivity and specificity of LAMP methods, 42 blood plasma samples and 21 tissue suspension samples (tonsils, spleen, lungs) were used. These samples were

preliminarily tested using two diagnostic PCR kits from different manufacturers designed to detect the ASF virus in biological material from animals. The results of detection of ASF virus DNA by two PCR kits gave similar results, 22 of 42 plasma samples and 11 of 21 tissue suspension samples contained ASF virus DNA.

Table 2: Evaluation of the specificity of LAMP against the ASF virus on RNA/DNA of various strains of viruses and microorganisms

Target DNA/RNA	Ct*/2, min
Classical swine fever virus	ND
Porcine circovirus 2	ND
Porcine epidemic diarrhea virus	ND
Porcine parvovirus	ND
Porcine reproductive and respiratory syndrome virus	ND
Rotavirus A	ND
Suid alphaherpesvirus 1	ND
Transmissible gastroenteritis virus	ND
mixed sample of bacterial DNA (Brucella suis; Campylobacter jejuni; Chlamydia suis; Escherichia coli; Haemophilus parasuis; Listeria monocytogenes; Pasterella multocida; Salmonella dublin; Shigella flexneri)	ND
Positive control, pPKO_ASF 10^5 copies/ml	13,0

Among 33 samples of biological material containing DNA of the ASF virus, 26 samples had a sufficient viral load with PCR threshold cycles Ct less than 35, 7 samples had a low content of ASF virus DNA in samples, PCR Ct more than 35.

The LAMP technique developed in this work makes it possible to detect ASF virus DNA in all samples with a sufficient viral load; the time for accumulation of fluorescence to the threshold level with LAMP is from 14 to 22 minutes (Figure 2).

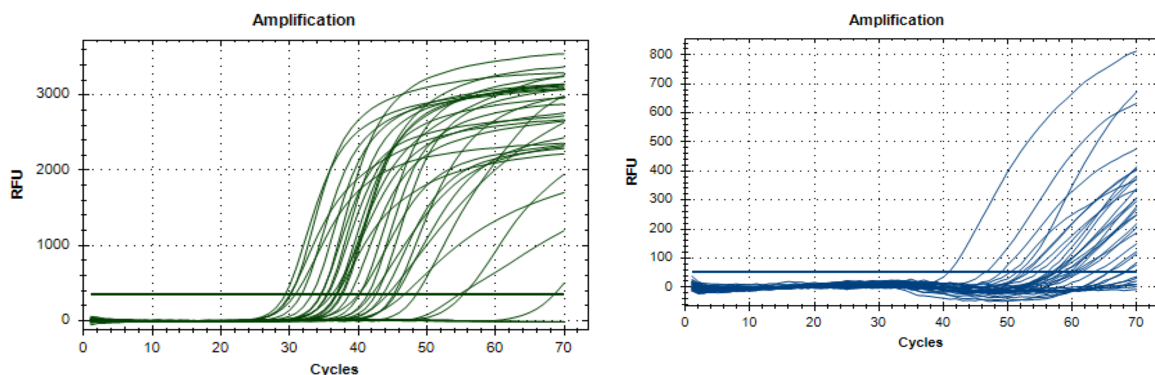

Figure 2: Diagnostic sensitivity of LAMP with fluorescent probe. Characterized blood plasma samples and tissue suspensions (by PCR) containing DNA of the ASF virus were studied, the reaction time was 35 minutes.

For samples with a low viral load (PCR Ct > 35), the LAMP probe method detected the virus in 6 (7) samples. All 30 PCR negative samples were identified correctly, even with an increase in the reaction time to 60 min, which indicates the high specificity of the developed LAMP method.

Table 3: Diagnostic characteristics of LAMP

Characteristic	Meaning	Confidence interval, p=95%
Specificity	100	88.43% - 100.00%
Sensitivity	97,0	84.24% - 99.92%
32 (out of 33) positive and 30 (out of 30) negative are identified correctly		

The diagnostic sensitivity of the real-time LAMP method with a probe is practically not inferior in sensitivity to PCR and is 97.0%. (Table 3).

4 Conclusion

The advantage of the test based on LAMP amplification using fluorescent probes stained with fluorophores is the ability to assess the efficiency of DNA extraction by co-amplification of control DNA in a multiplex reaction with ASF virus DNA, its high sensitivity and specificity.

The method proposed in the LAMP article for detecting the p72 gene of the ASF virus in the presence of an internal control sample of bacteriophage lambda DNA is an example of a rapid test that has good diagnostic characteristics, not inferior to PCR, a classical method of molecular diagnostics. The test also showed good diagnostic sensitivity in detecting the ASF virus from biological material samples - blood plasma and tissue suspension. The diagnostic sensitivity of LAMP was 97.0% (84.2 - 99.9; p=95), the specificity was 100% (88.43 - 100; p=95).

The developed LAMP method provides a significant reduction in the study time from 80-120 minutes required on average for PCR amplification to 35 minutes required for isothermal amplification.

5 Availability of Data and Material

Data can be made available by contacting the corresponding author.

6 Acknowledgement

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7 References

[1] Mori Y, Nagamine K, Tomita N, Notomi T. Detection of loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation. Biochemical and biophysical research communications. 2001;289(1):150-4.

[2] Tanner, N.A., Zhang, Y., Evans, T.C. Jr. (2015). Visual detection of isothermal nucleic acid amplification using pH-sensitive dyes. *Biotechniques*. 58(2):59-68.

[3] Wang D, Yu J, Wang Y, Zhang M, Li P, Liu M, Liu Y. Development of a real-time loop-mediated isothermal amplification (LAMP) assay and visual LAMP assay for detection of African swine fever virus (ASFV). Journal of virological methods. 2020;276:113775.

[4] Wang Y, Dai J, Liu Y, Yang J, Hou Q, Ou Y, Ding Y, Ma B, Chen H, Li M, Sun Y. Development of a potential penside colorimetric LAMP assay using neutral red for detection of african swine fever virus. Frontiers in microbiology. 2021;12:609821.

[5] Miao F, Zhang J, Li N, Chen T, Wang L, Zhang F, Mi L, Zhang J, Wang S, Wang Y, Zhou X. Rapid and sensitive recombinase polymerase amplification combined with lateral flow strip for detecting African swine fever virus. Frontiers in microbiology. 2019 May 15;10:1004.

[6] Yu LS, Chou SY, Wu HY, Chen YC, Chen YH. Rapid and semi-quantitative colorimetric loop-mediated isothermal amplification detection of ASFV via HSV color model transformation. Journal of

Microbiology, Immunology and Infection. 2021 Oct 1;54(5):963-70.

[7] Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. (2018). Molecular Biology and Evolution 35:1547-1549

[8] Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J. *J Mol Biol*. 1990; 215 (3): 403–10.

[9] Yaren O, Alto BW, Gangodkar PV, Ranade SR, Patil KN, Bradley KM, Yang Z, Phadke N, Benner SA. Point of sampling detection of Zika virus within a multiplexed kit capable of detecting dengue and chikungunya. BMC infectious diseases. 2017 Dec;17(1):1-3.

[10] MedCalc's Diagnostic test evaluation calculator https://www.medcalc.org/calc/diagnostic_test.php

[11] Lepej SZ, Poljak M. Portable molecular diagnostic instruments in microbiology: current status. Clinical Microbiology and Infection. 2020 Apr 1;26(4):411-20.

[12] Sereda AD, Makarov VV, Sachivkina NP, Strizhakov AA, Gnezdilova LA, Kuznetsov VI, Sturov NV, Zimina VN. Effectiveness of combined use: inactivated vaccines with immunostimulants on the in vivo model of Teschen disease. Advances in Animal and Veterinary Sciences. 2020;8(2):151-6.

[13] Belousova, R.V., Yarygina, E.I., Tretyakova, I.V. (2018). Virology and biotechnology. St. Petersburg, Lan Publishing House, 220 p.

[14] Tretyakova, I.V., Kalmykova, M.S., Yarygina, E.I. (2019). Cell culture is a universal model for culturing viruses. Vet*erinary medicine, zootechnics and biotechnology*. 4:30-36.

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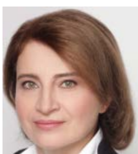

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