

# Isothermal Nucleic Acid Amplification System: An Update on Methods and Applications

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## Abstract

Polymerase chain reaction is the most commonly used nucleic acid amplification for the diagnosis of several infectious and non-infectious diseases of animal and humans. But, it requires high expensive instrumentation for amplification and analysis. There are several isothermal amplification methods, such as Nucleic Acid Sequence Based Amplification (NASBA), Strand Displacement Amplification (SDA), Isothermal Multiple Displacement Amplification (IMDA), Rolling Circle Amplification (RCA), Helicase-Dependent Amplification (HDA), Single Primer Isothermal Amplification (SPIA), signal-mediated amplification of RNA technology, circular helicase dependent amplification, Recombinase Polymerase Amplification (RPA), Polymerase Spiral Reaction (PSR) and Loop-mediated Isothermal Amplification (LAMP). These methods can be amplified at a constant temperature by a simple water bath or heating block without use of high cost equipment's like thermal cyclers and detection systems. The isothermal methods have been used for the detection of nucleic acids, proteins, enzymes and cancer cells. In this article, we reviewed the important isothermal amplification techniques and their applications in diagnosis and molecular biology.

**Keywords:** Isothermal amplification; Advantages; Disadvantages; Applications

## Introduction

Nucleic acid amplification is most commonly used for diagnosis, research, forensics, medicine and agriculture. There are different isothermal nucleic acid amplification methods have been invented. We describe here the important isothermal amplifications used for amplification of nucleic acid, including Nucleic Acid Sequence Based Amplification (NASBA) [1], Strand Displacement Amplification (SDA) [2], Isothermal Multiple Displacement Amplification (IMDA), Self-Sustained Sequence Replication Reaction (3SR) [3], Transcription-Based Amplification System (TAS) [4], Rolling Circle Amplification (RCA), Helicase-Dependent Amplification (HDA) [5], Single Primer Isothermal Amplification (SPIA) [6], Signal Mediated Amplification of RNA Technology (SMART), Circular Helicase Dependent Amplification (cHDA), Recombinase Polymerase Amplification (RPA) [7], and Loop-Mediated Isothermal Amplification (LAMP) [8] and their applications in diagnosis. Among these amplification methods, like RCR, LAMP and CPA can be amplified product at a constant temperature [9]. The most widely used nucleic acid-based amplification is the polymerase chain reaction (PCR) [10]. However, the application of PCR in the field is highly difficult as it requires thermo cyclers and highly expensive real-time cyclers. There are several nucleic acid-based amplification systems that have been invented without the need of thermocycler using simpler heating blocks and various detection methods (Table 1). There are several advancements in detection strategy applicable to these isothermal amplification formats in miniaturized systems [11] to enable them in quick and precision diagnosis.

## Nucleic Acid Sequence Based Amplification (NASBA)

NASBA was invented in 1991 [1] and modified with a further demonstration of Multiplex NASBA [12] and Duplex NASBA [13]. It is also called as Transcription Mediated Amplification (TMA) [14] and Self-Sustained Sequence Replication (3SR) [15]. This isothermal amplification is performed at a constant temperature of 41°C by three enzymes (i.e., RNase H, avian myeloblastosis virus reverse transcriptase, T7 DNA-dependent RNA polymerase (DdRp) [1]. NASBA mostly is used for the Amplification of mRNA, rRNA, tmRNA or genomic RNA [16] and for DNA amplification NASBA requires denaturation steps.

RNA products are detected by the gel electrophoresis, colorimetric assay (NASBA-ELISA), Fluorescence probes (real-time NASBA), and electrochemiluminescence [17], the lateral flow [18], Enzyme-Linked Gel Assay (ELGA) [19] and the electrochemical detection [20]. It has been used for detection of hepatitis C virus and human immunodeficiency virus-1 [21,22].

## Strand Displacement Amplification (SDA)

Strand Displacement Amplification (SDA) [2] is an isothermal nucleic acid-based amplification method. SDA performed by the ability of a restriction endonuclease to nick the unmodified strand of its target DNA and *Escherichia coli* DNA polymerase 1 (exo-Klenow) enzyme which elongates four primers in the reaction at high temperatures. During the amplification,  $10^9$  copies of DNA are produced in less than an hour. SDA also used for amplification of RNA by reverse transcriptase in RT-SDA format [23]. A major disadvantage of SDA is the inability to amplification of long target sequences. SDA is used for clinical diagnosis of diseases like chlamydia and gonorrhoea [24].

## Isothermal Multiple Displacement Amplification (IMDA)

IMDA is a highly sensitive strand displacement method, which amplifies the whole genome multiple primers by bacteriophage  $\phi$ 29 DNA polymerase enzyme at 30°C for 16-18 hours [25,26]. IMDA reaction can be directly carried out from crude samples [27]. It consists of two types of method, multiple strand displacement amplification and whole genome strand displacement amplification [28].

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Isothermal systems	PCR	NASBA	SDA	LAMP	RCA	SMART	HDA	SPIA
Name of enzyme(s) used	Taq DNA polymerase	-RNase H, avian myeloblastosis virus reverse transcriptase, T7 DNA-dependent RNA polymerase	<i>Escherichia coli</i> DNA polymerase 1, T7 RNA polymerase	Bst/Bsm DNA polymerase; Csa DNA polymerase; OmniAmp polymerase for both RNA/ DNA	Bacteriophage Ø29 DNA polymerase	Bst DNA Polymerase, T7 RNA polymerase and DNA polymerase	DNA helicase, exonuclease -deficient DNA polymerases	DNA polymerase, RNaseH, reverse transcriptase
No. of enzyme(s)	1	2-3	2	1	1	2-3	2	2-3
Time	2-3 h	within 2 h	16-18 h	Within 1 h	Within 1 h	2-3 h	2 h	3-4 h
Temp (s) °C	95, 55-60, 72	41	37	60-66	37	41	37, 60-65	45, 50
Denaturing agents	Heat	RNaseH	Restriction enzymes, Bumper primers	Betaine	Phage Ø29 DNA polymerase	RNaseH	helicase	RNaseH
Tolerance to crude samples	-	-	-	+	-	-	+	-
Primer design	simple	simple	complex	complex	simple	complex	simple	simple
Detection methods used	AGE	Auto-fluorescence By a common microplate reader; Molecular beacon fluorescence; Real-time fluorescence; Molecular beacon fluorescent probe technology	visual detection by fluorescent and metal ion indicator dyes; LFD	Optical detection of turbidity; visual detection by fluorescent and metal ion indicator dyes; LFD	Electrophoretic analysis; Fluorescence image; flow cytometry; Visual fluorescence atomic force microscopy; Real-time fluorescence; Laser scanning and image analysis	Real-time fluorescence Gel electrophoresis; real-time fluorescence	Real-time fluorescence Naked eye by an enzymatic process employing the dye Tetramethylbenzidine (TMB) Visual detection Electrochemical analysis	Real-time fluorescence Gel electrophoresis; real-time fluorescence

PCR: Polymerase Chain Reaction; HAD: Helicase-Dependent Amplification; LAMP: Loop-Mediated Isothermal Amplification; SDA: Strand Displacement Amplification; NASBA: Nucleic Acid Sequence Based Amplification; RCA: Rolling Circle Amplification; SMART: Signal-Mediated Amplification of RNA Technology; SPIA: Single Primer Isothermal Amplification; DNA: Deoxyribonucleic Acid; RNA: Ribonucleic Acid; Bst: *Bacillus stearothermophilus*; Taq: *Thermus Aquaticus*; AGE: Agarose Gel Electrophoresis

**Table 1:** Properties of different isothermal amplification methods and their detection strategy.

### Rolling Circle Amplification (RCA)

RCA is a highly specific isothermal method which extends a circle-hybridized primer by progressing around the circular DNA probe and it utilizes the bacteriophage Ø29 DNA polymerase enzyme for synthesis at a single temperature [29,30]. RCA suited for both cell-based and tissue-based assays. RCA is resistant to contamination and amplification errors. Because of the simplicity, accuracy and efficiency of RCA, makes RCA corrigible to miniaturization and automation in the high throughput analysis [31]. RCA also used for in situ or microarray hybridization assays.

### Helicase-Dependent Amplification (HAD)

HAD follows replication fork mechanism for amplification, which utilizes DNA helicase for unwinding activity [5] Helicase separates the duplex DNA into single-stranded DNA for *in vitro* amplification of target DNA. The amplification is extended by exonuclease deficient DNA polymerases at a single constant temperature. The HAD products are detected by gel electrophoresis, ELISA and real-time format [32]. Because of simplicity, processivity (10 kb/Binding) and high speed

(100 bp/s) in HAD, it provides several advantages over the other amplification method [33].

### Ramification Amplification Method (RAM)

RAM is a simple method which is similar to the “rolling circle” replication of bacteriophages *in vivo*. RAM amplifies a designed circular probe (Cprobe) ends which are joined together in juxtaposition by hybridization to a target DNA which elongates by a bacteriophage Ø29 DNA polymerase enzyme at 35°C by within 60 minutes [34]. There is no requirement of reverse transcriptase for RNA amplification. RAM can be applicable for the detection of DNA/RNA in Hematology, infectious disease, genetic disease, Pathology and Oncology. Because of the derivation of amplification power during strand displacement, primer extension, and multiple ramification (branching) points, this method is called ‘RAM’.

### Single Primer Isothermal Amplification (SPIA)

SPIA [6] utilizes single chimeric primer for the amplification of DNA. This Chimeric primer composed of ribonucleotides, deoxyribonucleotides, DNA polymerase and RNase H [14]. Ribo-SPIA

method used for amplification of RNA, especially linear mRNAs. SPIA is used for amplification of a large amount of nucleic acids from limiting biological samples [6]. The amplified products are detected by Bioanalyzer.

### Circular Helicase-Dependent Amplification (cHDA)

cHDA method is used for amplification of circular DNA template [35], which are amplified at a constant temperature of 25°C by T7 replication machinery (T7 sequence and T7 Gp2.5 SSB protein) [6,36].

### Signal-Mediated Amplification of RNA Technology (SMART)

SMART method based on signal amplification and it does not depend on copying of target sequences. The SMART method consists of two single-stranded oligonucleotide probes that are annealed to a specific target sequence and then it forms a three-way junction (3WJ) structure. The reaction is carried out by three enzymes like Bst DNA Polymerase, T7 RNA polymerase and DNA polymerase at a constant temperature of 41°C [6]. The amplicons are detected by ELOSA and real-time [37,38]. SMART used for detection of both DNA and RNA targets [39].

### Recombinase Polymerase Amplification (RPA)

RPA isothermal method amplifies target DNA sequences by using a DNA polymerase, recombinase and DNA-binding proteins at a temperature of 37-42°C. The amplified products are detected by the fluorophore, quencher groups and microfluidic devices [40].

### Loop-Mediated Isothermal Amplification (LAMP)

The LAMP is a simple, novel and rapid isothermal amplification method. LAMP assay can be amplified at a constant temperature by water bath/heat block by using Bst polymerase large fragment and it is used for development of POCT (point of care testing) in a field [41]. The LAMP uses four to six primers for amplification. The LAMP assay can also use to quantify the amount of amplified DNA [42]. The reactions are tolerant against inhibiting substances in samples [43]. The LAMP assay amplifies few copies of Non-denatured DNA into billion copies [8,41]. The LAMP is used for detection of nucleic acids in bacteria, viruses and parasites, genotyping and LAMP based SNP typing. The amplified products are detected by closed-tube visual methods, gel electrophoresis, by DNA intercalating dyes and metal-ion indicator dyes, by real-time turbid meter and later flow assay. RT-LAMP is used for RNA amplification.

### Polymerase Spiral Reaction (PSR)

PSR is a novel isothermal nucleic acid amplification method requiring only one pair of primers and one enzyme like PCR with high specificity, efficiency, and rapidity under isothermal conditions [44]. PSR assay can be monitored continuously in a real-time turbid meter instrument or visually detected using fluorescent dye (SYBR Green I) or metal ion indicator dyes (HNB and Calcein) and other detection methods that have been used for LAMP technology. PSR could be finished within 1 h with a high sensitivity (up to 10<sup>9</sup> copies) and high specificity to detect the target. The PSR method exploits the advantages of PCR in which only one pair of primers is needed and isothermal amplification techniques like LAMP assay. This novel emerging nucleic acid amplification system has been successfully applied in rapid and sensitive detection of *Candida albicans* in clinical blood samples [45], canine parvovirus 2 genomic DNA (CPV-2) in fecal samples [46] and African swine fever virus in pigs and wild boars using modified method called as Polymerase Cross Linking Spiral Reaction (PCLSR).

## Emerging Trends and Methods of Isothermal Amplification System

### • Beacon Assisted Detection Amplification (BADAMP)

The BADAMP method can be used to detect and amplify short DNA sequences (22 bp) [47,48].

### • Hairpin fluorescence probe assisted isothermal amplification

The hairpin fluorescence probe assisted isothermal amplification method used to detect and amplify the microRNA (22 nucleotides). This method is similar to that of the BADAMP [49].

### • Exponential amplification reaction (EXPAR)

EXPAR is similar to SDA. EXPAR is used for characterizing polymorphic sites in genomic DNA and also to detect and amplify short oligonucleotides (8-16 bp) [50].

### • Exonuclease III-induced cascade two-stage isothermal amplification-mediated zinc(II)-protoporphyrin IX/G-quadruplex supramolecular fluorescent nanotags

This method is used for early diagnosis of gene-related diseases [51].

### • Hinge-Initiated Primer-dependent Amplification of Nucleic Acids (HIP)

HIP mechanism used for amplification DNA by using a linker modification [52].

## Conclusions and Future Perspectives

The broad applications of isothermal applications include detection of nucleic acid like DNA, RNA, long RNA, SNPs and miRNA, DNA methylation, proteins and enzymes, cancer cells and pathogens, small molecules and metal-ions, used for sequencing, in situ and intracellular analysis [53]. We reviewed the isothermal nucleic acid amplification techniques that have been successfully implemented for the rapid and simple detection of nucleic acids [54,55]. The above mechanism and applications can be used as valuable diagnostic amplification techniques for the detection of nucleic acids in the field. These isothermal amplification systems meet the guidelines proposed by the World Health Organization for developing diagnostic techniques, namely, ASSURED (affordable, sensitive, specific, user friendly, robust and rapid, equipment free and deliverable) and provide a convenient and cost-effective alternative for clinical screening, on-site diagnosis and primary quarantine purposes [56-59]. In the future, these methods may be acceptable as PCR systems to replace them as an alternate due to their applicability in resource limited field diagnostic settings of developing world.

## References

1. Compton J (1991) Nucleic acid sequence-based amplification. *Nature* 350: 91-92.
2. Walker GT, Fraiser MS, Schram JL, Little MC, Nadeau JG, et al. (1992) Strand displacement amplification-an isothermal, in vitro DNA amplification technique. *Nucleic Acids Res* 20: 1691-1696.
3. Fahy E, Kwok DY, Gingeras TR (1991) Self-sustained sequence replication (3SR): an isothermal transcription-based amplification system alternative to PCR. *PCR Methods Appl* 1: 25-33.
4. Kwok DY, Davis GR, Whitfield KM, Chappelle HL, DiMichele LJ, et al. (1989) Transcription-based amplification system and detection of amplified human immunodeficiency virus type 1 with a bead-based sandwich hybridization format. *Proc Natl Acad Sci USA* 86: 1173-1177.

5. Vincent M, Xu Y, Kong H (2004) Helicase dependent isothermal DNA amplification. *EMBO Rep* 5: 795-800.
6. Kurn N, Chen P, Heath JD, Kopf-Sill A, Stephens KM, et al. (2005) Novel isothermal, linear nucleic acid amplification systems for highly multiplexed applications. *Clin Chem* 51: 1973-1981.
7. Piepenburg O, Williams CH, Stemple DL, Armes NA (2006) DNA Detection Using Recombination Proteins. *PLoS Biol* 4: 204.
8. Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, et al. (2000) Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res* 28: e63.
9. Murray L, Edwards L, Tuppurainen ES, Bachanek-Bankowska K, Oura CA, et al. (2013) Detection of Capripoxvirus DNA using a novel loop-mediated isothermal amplification assay. *BMC Vet Res* 9: 90.
10. Mullis KB, Faloona FA (1987) Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods Enzymol* 155: 335-350.
11. Tröger V, Niemann K, Gärtig C (2015) Isothermal Amplification and Quantification of Nucleic Acids and its Use in Microsystems. *J Nanomed Nanotechnol* 6: 282.
12. Van Deursen PBH, Gunther AW, Spaargaren-van Riel CC, van den Eijnden MMED, Vos LH, et al. (1999) A novel quantitative multiplex NASBA method: application to measuring tissue factor and CD14 mRNA levels in human monocytes. *Nucleic Acids Res* 27: e15.
13. Jean J, Blais B, Darveau A, Fliss I (2002) Rapid detection of human rotavirus using colorimetric nucleic acid sequence-based amplification (NASBA)-enzyme-linked immunosorbent assay in sewage treatment effluent. *FEMS Microbiol Lett* 210: 143-147.
14. Gill P, Ghaemi A (2008) Nucleic acid isothermal amplification technologies - A review. *Nucleosides, Nucleotides and Nucleic Acids* 27: 224-243.
15. Guatelli JC, Whitfield K, Kwoh D, Barringer KJ, Richman DD, et al. (1990) Isothermal, in vitro amplification of nucleic acids by a multienzyme reaction modeled after retrovirus replication. *Proc Natl Acad Sci USA* 87: 1874-1878.
16. Deiman B, van Aarle P, Sillekens P (2002) Characteristics and applications of nucleic acid sequence-based amplification (NASBA). *Mol Biotechnol* 20: 163-179.
17. Shan S, Ko LS, Collins RA, Wu Z, Chen J, et al. (2003) Comparison of nucleic acid-based detection of avian influenza H5N1 with virus isolation. *Biochem Biophys Res Commun* 302: 377-383.
18. Connelly JT, Nugen SR, Borejsza-Wysocki W, Durst RA, Montagna RA, et al. (2008) Human pathogenic *Cryptosporidium* species bioanalytical detection method with single oocyst detection capability. *Anal Bioanal Chem* 391: 487-495.
19. Samuelson A, Westmoreland D, Eccles R, Fox JD (1998) Development and application of a new method for amplification and detection of human rhinovirus RNA. *J Virol Methods* 71: 197-209.
20. Nugen SR, Asiello PJ, Connelly JT, Baeumner AJ (2009) PMMA biosensor for nucleic acids with integrated mixer and electrochemical detection. *Biosens Bioelectron* 24: 2428-2433.
21. Guichón A, Chiparelli H, Martínez A, Rodríguez C, Trento A, et al. (2004) Evaluation of a new NASBA assay for the qualitative detection of hepatitis C virus based on the NucliSense® Basic Kit reagents. *J Clin Virol* 29: 84-91.
22. Bremer J, Nowicki M, Beckner S, Brambilla D, Cronin M, et al. (2000) Comparison of two amplification technologies for detection and quantitation of human immunodeficiency virus type 1 RNA in the female genital tract. *J Clin Microbiol* 38: 2665-2669.
23. Hellyer TJ, DesJardin LE, Teixeira L, Cave MD, Eisenach KD, et al. (1999) Detection of viable *Mycobacterium tuberculosis* by reverse transcriptase-strand displacement amplification of mRNA. *J Clin Microbiol* 37: 518-523.
24. Chan EL, Brandt K, Olien K, Antonishyn N, Horsman GB, et al. (2000) Performance characteristics of the Becton Dickinson ProbeTec System for direct detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in male and female urine specimens in comparison with the Roche Cobas System. *Arch Pathol Lab Med* 124: 1649-1652.
25. Hawkins TL, Dettler JC, Richardson PM (2002) Whole genome amplification-Applications and advances. *Curr Opin Biotechnol* 13: 65-67.
26. Hughes S, Armeson N, Done S, Squire J (2005) The use of whole genome amplification in the study of human disease. *Prog Biophys Mol Biol* 88: 173-189.
27. Morisset D, Stebih D, Cankar K, Zel J, Gruden K, et al. (2008) Alternative DNA amplification methods to PCR and their application in GMO detection: Review. *Eur Food Res Technol* 227: 1287-1297.
28. Luthra R, Medeiros LJ (2004) Isothermal multiple displacement amplification; a highly reliable approach for generating unlimited high molecular weight genomic DNA from clinical specimens. *J Mol Diag* 6: 236-242.
29. Cho EJ, Yang L, Levy M, Ellington AD (2005) Using a deoxyribozyme ligase and rolling circle amplification to detect a non-nucleic acid analyte, ATP. *J Am Chem Soc* 127: 2022-2023.
30. Demidov VV (2002) Rolling-circle amplification in DNA diagnostics: The power of simplicity. *Expert Rev Mol Diagn* 2: 542-548.
31. Gusev Y, Sparkowski J, Raghunathan A, Ferguson H, Montano J, et al. (2001) Rolling circle amplification: A new approach to increase sensitivity for immunohistochemistry and flow cytometry. *Am J Pathol* 159: 63-69.
32. Gill P, Tehrani HA, Ghaemi A (2007) Molecular detection of *Mycobacterium tuberculosis* by tHDAELISA DIG detection system. *Int J Antimicrob Agents* 29: 570-571.
33. Jeong YJ, Park K, Kim DE (2009) Isothermal DNA amplification in vitro: The helicasedependent amplification system. *Cell Mol Life Sci* 66: 3325-3336.
34. Beals TP, Smith JH, Nietupski RM, Lane JD (2010) A mechanism for ramified rolling circle amplification. *BMC Mol Biol* 11: 94.
35. Xu Y, Kim HJ, Kays A, Rice J, Kong H, et al. (2006) Simultaneous amplification and screening of whole plasmids using T7 bacteriophage replisome. *Nucleic Acids Res* 34: e98.
36. Karami A, Gill P, Motamedi MH, Saghafia M (2011) A review of the current isothermal amplification techniques: Applications, advantages and disadvantages. *J Global Infect Dis* 3: 293-302.
37. Tyagi S, Kramer FR (1996) Molecular beacons: Probes that fluoresce upon hybridization. *Nat Biotechnol* 14: 303-308.
38. Tyagi S, Bratu DP, Kramer FR (1998) Multicolor molecular beacons for allele discrimination. *Nat Biotechnol* 16: 49-53.
39. Wharam SD, Marsh P, Lloyd JS, Ray TD, Mock GA, et al. (2001) Specific detection of DNA and RNA targets using a novel isothermal nucleic acid amplification assay based on the formation of a three-way junction structure. *Nucleic Acids Res* 29: e54.
40. Zanolli LM, Spoto G (2013) Isothermal Amplification Methods for the Detection of Nucleic Acids in Microfluidic Devices. *Biosensors* 3: 18-43.
41. Nagamine K, Watanabe K, Ohtsuka K, Hase T, Notomi T, et al. (2001) Loop-mediated isothermal amplification reaction using a non-denatured template. *Clin Chem* 47: 1742-1743.
42. Fujino M, Yoshida N, Yamaguchi S, Hosaka N, Ota Y, et al. (2005) A simple method for the detection of measles virus genome by loop-mediated isothermal amplification (LAMP). *J Med Virol* 76: 406-413.
43. Kaneko H, Kawana T, Fukushima E, Suzutani T (2007) Tolerance of loop-mediated isothermal amplification to a culture medium and biological substances. *J Biochem Biophys Methods* 70: 499-501.
44. Liu W, Dong D, Yang Z, Zou D, Chen Z, et al. (2015) Polymerase spiral reaction (PSR): A novel isothermal nucleic acid amplification method. *Sci Rep* 5: 12723-12730.
45. Jiang X, Dong D, Bian L, Zou D, He X, et al. (2016) Rapid detection of *Candida albicans* by polymerase spiral reaction Assay in clinical blood samples. *Front Microbiol* 7: 916.
46. Gupta V, Chakravarti S, Chander V, Majumder S, Bhat SA, et al. (2018) Polymerase spiral reaction (PSR): a novel, visual isothermal amplification method for detection of canine parvovirus 2 genomic DNA. *Arch Virol* 162: 1995-2001.
47. Connolly AR, Trau M (2010) Isothermal Detection of DNA by Beacon-Assisted Detection Amplification. *Angew. Chem Int Ed Engl* 49: 2720-2723.
48. Connolly AR, Trau M (2011) Rapid DNA detection by beacon-assisted detection amplification. *Nat Protoc* 6: 777-778.
49. Ma C, Liu S, Shi C (2014) Ultrasensitive detection of microRNAs based on hairpin fluorescence probe assisted isothermal amplification. *Biosens Bioelectron* 58: 57-60.



50. Van Ness J, Van Ness LK, Galas DJ (2003) Isothermal reactions for the amplification of oligonucleotides. *Proc Natl Acad Sci USA* 100: 4504-4509.
51. Xue Q, Lv Y, Zhang Y (2014) Ultrasensitive fluorescence detection of nucleic acids using exonuclease III-induced cascade two-stage isothermal amplification-mediated zinc (II)-protoporphyrin IX/G-quadruplex supramolecular fluorescent nanotags. *Biosens Bioelectron* 61: 351-356.
52. Fischbach J, Frohme M, Glökler J (2018) Hinge-initiated Primer-dependent Amplification of Nucleic Acids (HIP) – A New Versatile Isothermal Amplification Method. *Scientific Reports* 7: 7683.
53. Zhao Y, Chen F, Li Q (2015) Isothermal Amplification of Nucleic Acids. *Chem Rev* 115: 12491-12545.
54. Banér J, Nilsson M, Mendel-Hartvig M (1998) Signal amplification of padlock probes by rolling circle replication. *Nucleic Acids Res.* 26: 5073-5078.
55. Fakruddin M, Mannan KS, Chowdhury A (2013) Nucleic acid amplification: Alternative methods of polymerase chain reaction. *J Pharm Bioall Sci* 5: 245-452.
56. Van Der Pol B, Ferrero DV, Buck-Barrington L (2001) Multicenter evaluation of the BDProbeTec ET System for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urine specimens, female endocervical swabs, and male urethral swabs. *J Clin Microbiol* 39: 1008-1016.
57. Van Gemen B, van Beuningen R, Nabbe A (1994) A one-tube quantitative HIV-1 RNA NASBA nucleic acid amplification assay using electrochemiluminescent (ECL) labelled probes. *J Virol Methods* 49: 157-168.
58. Walker GT (1993) Empirical aspects of strand displacement amplification. *PCR Methods Appl* 3: 1-6.
59. Wozniakowski G, Fraczyk M, Kowalczyk A, Pomorska-Mol M, Niemczuk K, et al. (2018) Polymerase cross-linking spiral reaction (PCLSR) for detection of African swine fever virus (ASFV) in pigs and wild boars. *Sci Rep* 7: 42903.