Supporting information

A novel exonuclease-assisted isothermal nucleic acid amplification with ultrahigh specificity mediated by full-length *Bst* DNA polymerase

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Supplementary Table

Table S1. The sequencing data of both large and small product of the rotavirus A using Exo-NAT method

Item	Sequences
The large product (207bp)	TCGACAACATGTACTTATTGAATGCCAAAATCTA TTGGTAGGAGTGAACAGTACATTTCACCGGATGC AGAAGCATTCAATAAGTACATGTTGTCGAAGTCT CCAGAGGATATTGGACCATCTGATTCTGCTTCAA ACGATCCACTCACCAGCTTTTCGATTAGATCGAA TGCAGTTAAGACAAGATGGTCCAATATCCTCTGG AGA
The small product (155bp)	TTGAATGCCAAAATCTATTGGTAGGAGTGAACAG TACATTTCACCGGATGCAGAGGCATTCAATAAGT ACATGTTGTCGAAGTCTCCAGAGGATATTGGACC ATCTTGTCTTAACTGCATTCGATCTAATCGAAAA GCTGGTGAGTGGATCGTTA

Experimental section

1. Materials

Full length *Bst* DNA polymerase (M0328) and large fragment *Bst* DNA polymerase (M0275) were purchased from the New England Biolabs. The electrophoresis kit was purchased from the Shanghai Sangon Biotech. SYTO-9 green fluorescent nucleic acid stain (S34854) and UltraPure™ DNase/RNase-free distilled water (10977015) were purchased from the ThermoFisher Scientific. The amplification process and the fluorescent signal were preformed and collected on Line Gene 9640, Hangzhou Bioer Co. Ltd.

The clinical faeces samples were collected from the children diarrhea patients, including rotavirus A (RV, n=10), astrovirus (AS, n=10), adenovirus (AD, n=10) and other bacterial pathogens (n=10) infection, in the clinical laboratory of the children's hospital, Fudan University. The informed consults were received from all subjects in this study. The total nucleic acid was extracted from all samples using a magnetic bead nucleic acid extraction kit and followed by reverse transcription to cDNA using a commercialized kit (Takara R036A). The clinical raw samples were stored at -80^{o}C and the cDNA was stored at -20 ^{o}C until the use.

Plasmid samples containing the highly conversed sequences of the three virus were synthesized by the Shanghai Sangon Biotech and series diluted to serve the detection limit assay.

2. The amplification system

The primers' sequences for Exo-NAT analysis of rotavirus A, astrovirus and adenovirus were listed in table S2, S3 and S4 respectively. All primers were synthesized by ThermoFisher Scientific followed by PAGE purification. The exactly concentrations of each components of the Exo-NAT reaction system were listed in table S5. The Exo-NAT reactions were conduct in 65^{o}C for 90 minutes, the fluorescence signal was collected at an interval of one minute. Followed by the melting curve analysis, from 60 ^{o}C to 95 ^{o}C at an interval of 0.2 ^{o}C.

3. Electrophoresis

The amplification products of both Exo-NAT and classic LAMP method were diluted 3 times and mixed with 6×loading buffer (Sangon, B540084) for electrophoresis using 1.5% agarose (Biowest, 111860) in 120V for 40 minutes under 1×TAE buffer (Sangon, B548101). The gel was stained using ethidium bromide (Sangon, A500328) and the results were obtained under a gel imaging system (Tanon, 4100).

4. Detection performance when mixing the three primers sets in a single tube

To verify whether the present Exo-NAT method has the potential to realize multiplex detection with the same high specificity, we try to mix the triple primer sets together to analyze 10 positive samples for each virus respectively and 10 bacteria pathogens (including *clostridium difficile*, *campylobacter jejuni*, *salmonella enteritidis*, *campylobacter coli* and *salmonella typhimurium* positive samples) as negative control. Then the detection limits were also conduct when mixing triple primer sets using the plasmid samples for each virus respectively.

Table S2. The primer sequences targeting the rotavirus A genes

Item	Sequences
Forward outer primer	CTACAACGTCAACTCTTTCTG
Reverse outer primer	AATCCATAGACACGCCAG
Forward inner primer	CGACAACATGTACTTATTGAATGCCAAAATCTATTGGTA GGAGTGAACA
Reverse inner primer	TCTCCAGAGGATATTGGACCATCTTGTCTTAACTGCATT CGATCT

Table S3. The primer sequences targeting the astrovirus genes

Item	Sequences
Forward outer primer	AATAACAATGGCAATTTAGCAC
Reverse outer primer	TGGTGCCAATAAAAACTGTT
Forward inner primer	AGACCACGTATCTGGCTCACTTGGCATATCTTCTTGTGC T
Reverse inner primer	GCACGCCTGTTTGACACTCACCTACAAGTTAGTATGACA ACAA

Table S4. The primer sequences targeting the adenovirus genes

Item	Sequences
Forward outer primer	AAGACAAAACGGCGTGCT
Reverse outer primer	GCTTACGGATTCCCAACAGA
Forward inner primer	TTGGTTACCGGGTCCCAACCAGAAAGCGACATAGGGGTGA
Reverse inner primer	CTTGTCATGCCAGGCGTGTACAGCGTAAAATCCACTCCGCA

Table S5. The concentration of each components of our new Exo-NAT method

Components	Final concentration or amount
Reaction Buffer	1X
dNTPs mixture (25mM)	1.4mM each
Each inner primer (100mM)	1.6μM each
Each outer primer (100mM)	0.2μM each
Full length *Bst* DNA polymerase	1μL
SYTO-9 (5mM)	0.5μM
Nucleic acid template	2μL
Ultra-pure water	Supplement the rest to 25μL

Supplementary Figures and Discussion

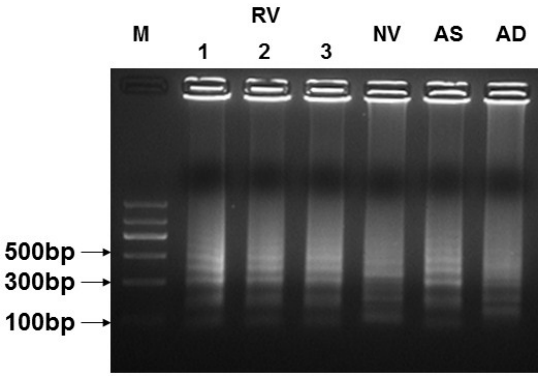

Figure S1. The amplicons distribution pattern using classic LAMP method. RV, AS, AD refers to rotavirus A, astrovirus and adenovirus respectively. The results showed that serious non-specific amplification occurred during classic LAMP.

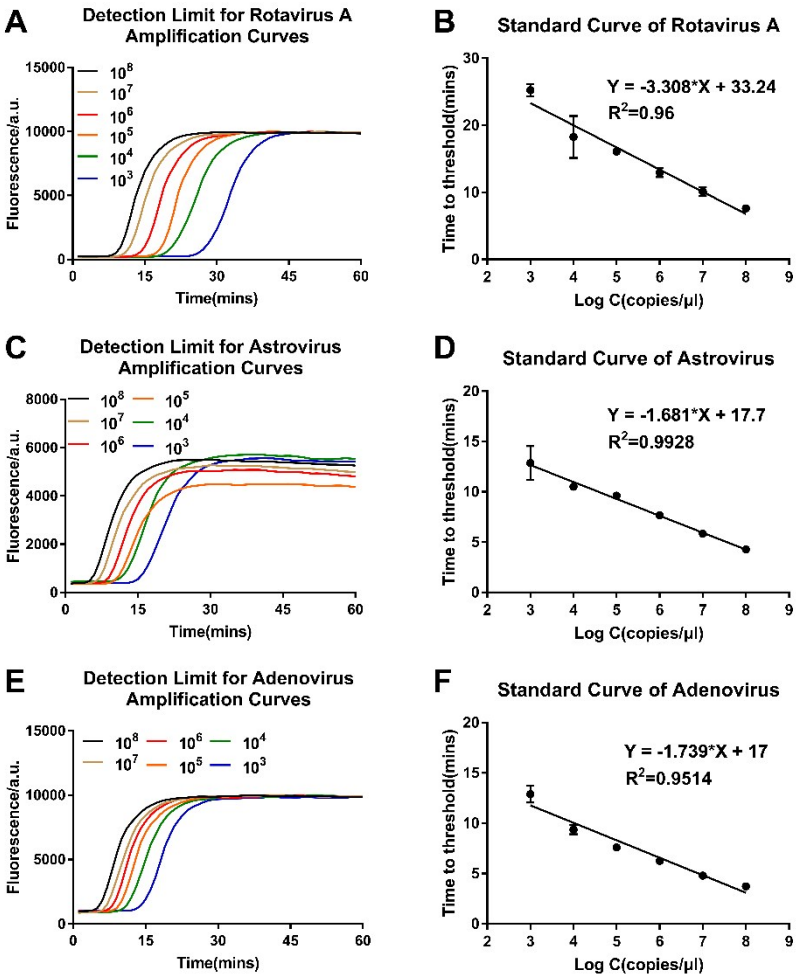

Figure S2. The detection limit using classic LAMP method for rotavirus A, astrovirus and adenovirus respectively.

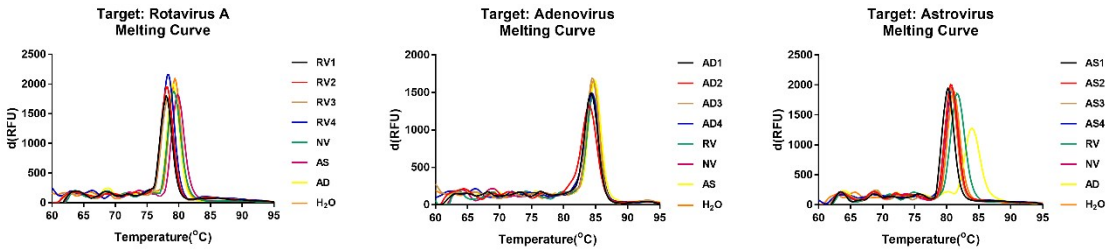

Figure S3. The specificity of the classic LAMP-melting curve analysis for the rotavirus A, adenovirus and astrovirus, respectively.

To emphasize the benefit of our Exo-NAT method, we also selected the most popular isothermal amplification method, LAMP, to make a comparison. The three virus positive samples were also detected using the classic LAMP method and the results were shown in Figure S3. It obviously suggested the LAMP method could not separate positive and negative samples even using melting curve. The length of the LAMP primers was also quiet long and the concentration of primers was quiet high, meanwhile the polymerase used in LAMP has an ultrahigh efficiency for the dNTPs incorporation and lacking the proof reading property, these factors coupled with the primer-self annealing and extending probably cause the signals in negative samples was very similar with positive samples. In addition, the classic LAMP products were very complex which was ladder model (Figure S1) and also bring difficult to the melting curve analysis. Furthermore, it could be reasonable speculation that the non-specific application will more serious under the triple primer sets mixture system when using LAMP, which indicated that the multiplex detection may also difficult to realize in classic LAMP. In addition, the detection limits of classic LMAP method were also higher than our present Exo-NAT method, indicating the less sensitivity of classic LAMP method. However, it needs to be noticed that the primers for our new Exo-NAT method still need be designed and optimized previously.