Supporting information

Sequence-Specific Probe Mediated Isothermal Amplification for the Single-Copy Sensitive Detection of Nucleic Acid

Xin Ye[a], Xueen Fang[*a], Yang Li[bc], Lijuan Wang[bc], Xinxin Li[bc] and Jilie Kong[*a]

a. Department of Chemistry and Institutes of Biomedical Sciences, Fudan University, Shanghai 200433, P.R. China.

b. Shanghai Suchuang Diagnostic Products Co., Ltd., Shanghai 201318, P.R.China

c. Shanghai Suxin Biotechnology Co. Ltd., Shanghai 201318, P.R.China

* Correspondence should be addressed to X.E.F. (E-mail:fxech@fudan.edu.cn) or J.L.K. (E-mail:jlkong@fudan.edu.cn).

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Experimental

Table S1. The sequences of the circular fluorescent probes targeting rotavirus and astrovirus

Target	Item	Sequences
Rotavirus	Forward	FAM-CGACAACATG/iTAMdT/ACTTATTGAATGCCAAAATCTATTGGTAGGAGTGAACA
	Reverse	FAM-TCTCCAGAGGA/iTAMdT/ATTGGACCATCTTGTCTTAACTGCATTCGATCT
Astrovirus	Forward	HEX-AGACCACG/iBHQ1dT/ATCTGGCTCACTTGGCATATCTTCTTGTGCT
	Reverse	HEX-GCACGCC/iBHQ1dT/GTTTGACACTCACCTACAAGTTAGTATGACAACAA

As mentioned in Figure 1C, two fluorescent-labeled probes and a single fluorescent-labeled probe were used. Here the two fluorescent-labeled probes mean both forward and reverse CFP targeting rotavirus were labeled with FAM in the 5' end. Meanwhile, a single fluorescent-labeled probe means just forward CFP targeting rotavirus were labeled with FAM in its 5' end and the reverse CFP didn't label with FAM.

Supplementary Results and Discussion

Firstly, the concentrations of circular fluorescent probes (Figure S1) were optimized. The results show that using 1.2 μM forward and reverse CFP were most suitable for CFPA. At the same time, too high or too low probe concentration will partially inhibit the reaction. There are two amplification curves in the following results (Figure S1-S4), which generated from the duplicate experiment and ensure the accuracy of the experiments.
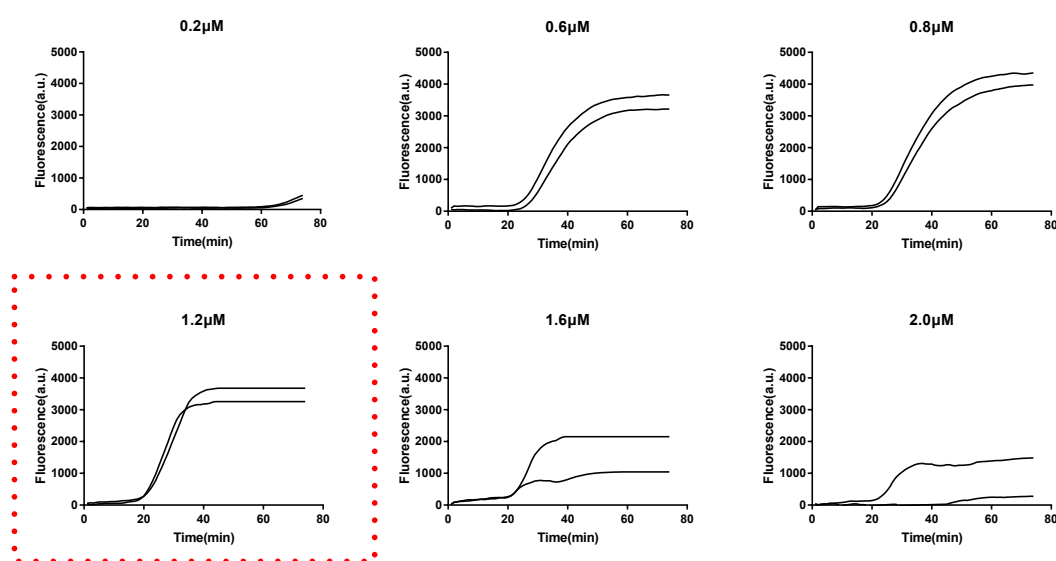

Figure S1. Optimization of the circular fluorescent probe concentration.

Next, we determined which DNA polymerase has the best performance and the results in Figure S2 show that the *Bst* 2.0 warmstart DNA polymerase has the fastest response time which is the most suitable enzyme for CFPA. When using *Taq* DNA polymerase, the reaction was unable to amplify for lacking the strand replacement capability.
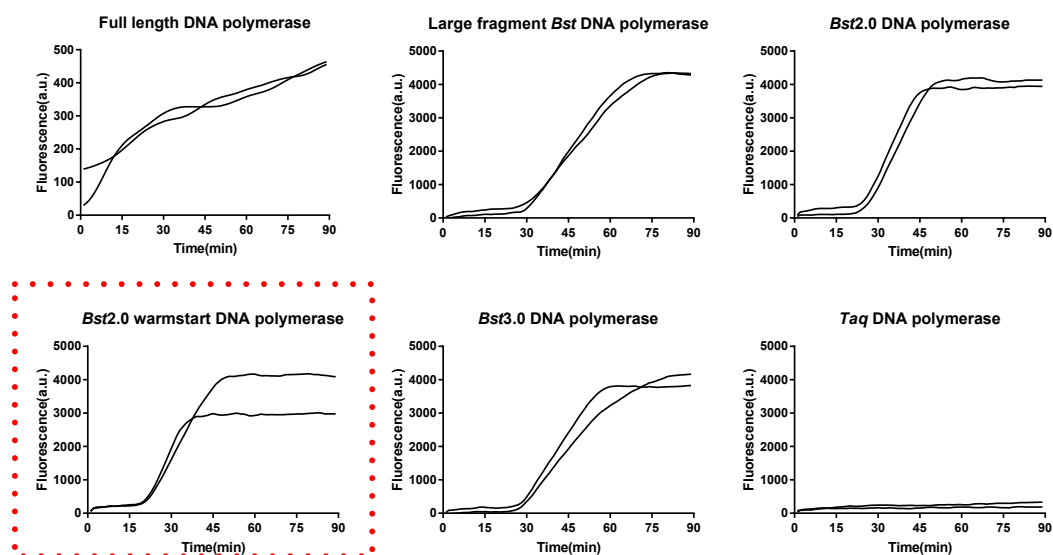

Figure S2. Optimization of the DNA polymerase.

Then, the amount of FEN1 (Figure S3) was optimized. When using different concentrations, the amplification effect was similar. Based on the time to threshold, 0.6U FEN1 was determined as the optimal condition.
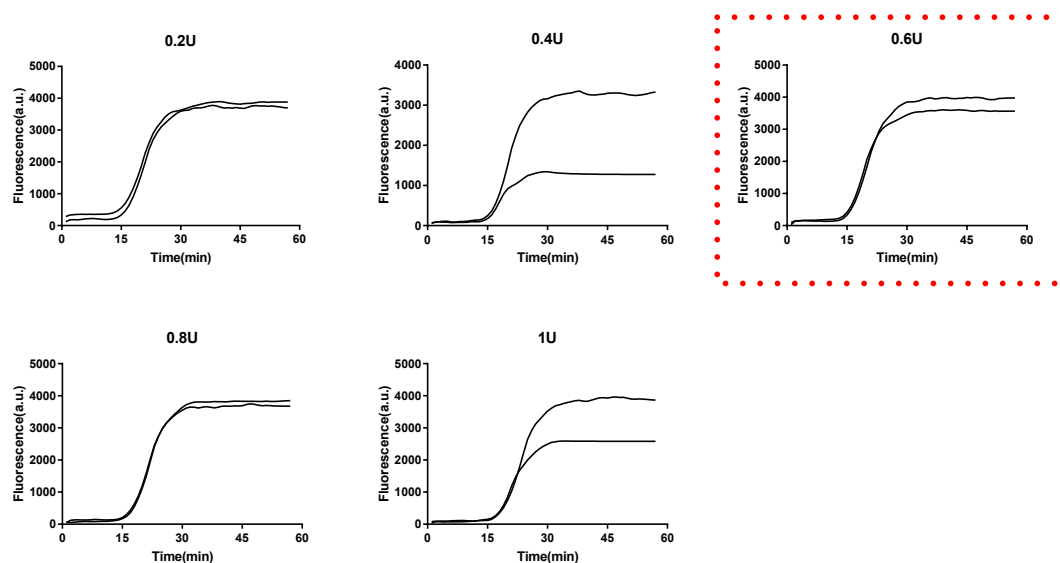

Figure S3. Optimization of the FEN1 amount.

Last, we also choose the best reaction temperature based on the time to threshold (Figure S4). The results showed 63°C has the smaller Tt value which means the reaction proceed faster under this condition.
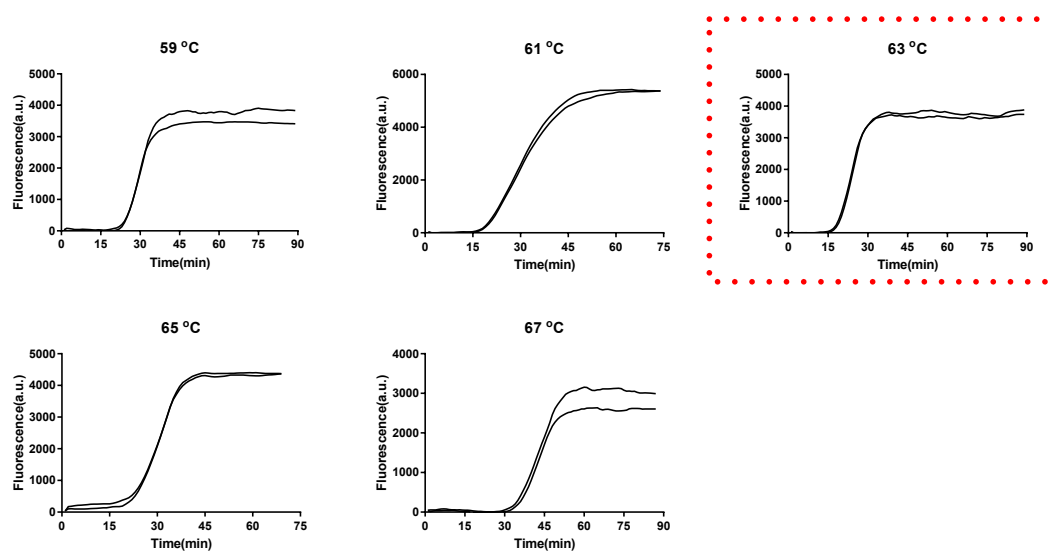

Figure S4. Optimization of the reaction temperature.

The specificity, sensitivity, detection limit and linearity of the CFPA method when detecting astrovirus cDNA samples (Figure S5), which further supported the ability of CFPA in testing DNAs.
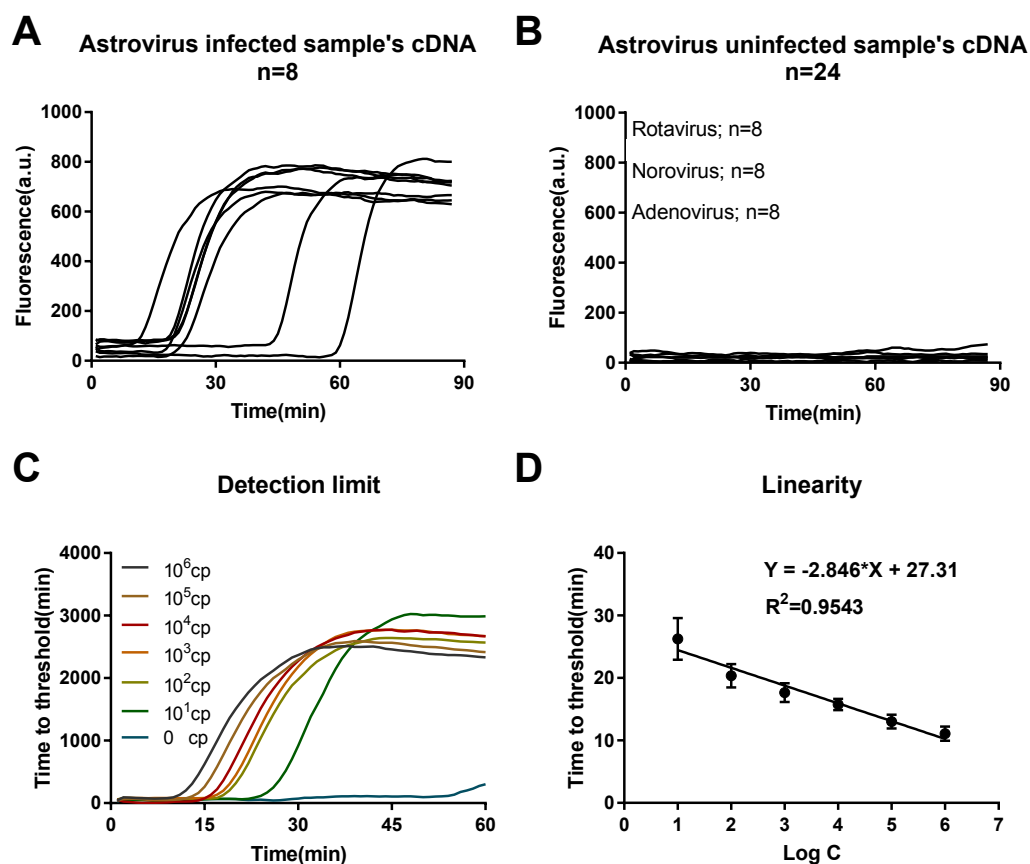
A Astrovirus infected sample's cDNA
n=8

B Astrovirus uninfected sample's cDNA
n=24

C Detection limit

D Linearity

$Y = -2.846*X + 27.31$
$R^2=0.9543$

Figure S5. The specificity, sensitivity, detection limit and linearity of the CFPA method for the cDNA of astrovirus (A–D). The fluorescence amplification curves when detecting astrovirus cDNA from infected (A) and uninfected (B) samples; (C) the fluorescence amplification curves of serially diluted cDNA (from $1\times10^7–1\times10^1$ copies); (D) the linearity between the Tt value and logarithmic value of astrovirus plasmid concentrations.

The detection performance of CFPA for astrovirus RNA samples, which was a kind of single-stranded RNA. The results (Figure S6) further proved the ability of CFPA in testing RNAs.
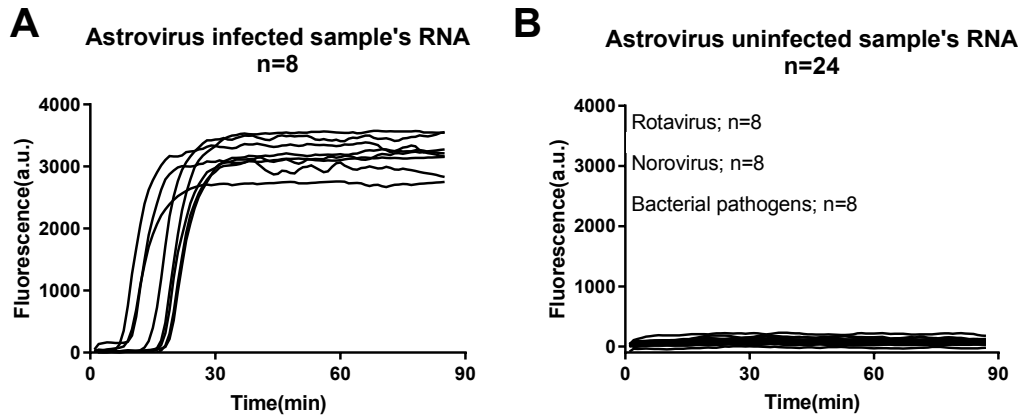

Figure S6. The specificity and sensitivity of the CFPA method for the direct detection of RNA. The templates were astrovirus -infected RNA (A) and uninfected RNA (B) samples.

The classic LAMP method was also used to diagnosis rotavirus infection in the 174 clinical samples. Table S2 show the results which clearly indicated the poorer diagnosis ability of LAMP when compared with the present CFPA method.

Table S2. Results of clinical trials of the comparison between the LAMP method and PCR

		PCR		
		Positive	Negative	Total
LAMP	Positive	90	33	123
	Negative	6	45	51
	Total	96	78	174