

Rapid detection and differentiation of Influenza A and B viral RNA using a novel isothermal nucleic acid amplification method, RT-SIBA®

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BACKGROUND

Timely diagnosis of influenza infection is important in infection control and prevention of inappropriate use of antibiotics. Rapid influenza tests that produce results in less than 30 min have become available in recent years. However, they are mainly immunoassays and often show sub-optimal test sensitivity. Molecular methods, such as RT-PCR, offer significant advantages in test sensitivity but often are time-consuming and require heavy instrumentation. Isothermal nucleic acid amplification methods that require only small, battery-operated instruments enable molecular diagnostics to be performed within point-of-care settings. We developed a novel isothermal nucleic acid amplification method called Reverse Transcriptase Strand Invasion Based Amplification (RT-SIBA®). Influenza A and B RT-SIBA assays were developed to demonstrate feasibility of RT-SIBA for rapid detection of viral RNA targets.

METHODS

We previously described the ability of SIBA® to detect DNA targets with high analytical sensitivity and specificity [1]. The mechanistic principles of the SIBA reaction are depicted in Figure 1. In this study, we developed a variant of the SIBA method, RT-SIBA, which includes a one-step reverse transcription of RNA into cDNA and simultaneous amplification and detection of cDNA under isothermal reaction conditions. Performance of RT-SIBA influenza A and B assays was compared to the previously published Centers for Disease Control and Prevention (CDC) real-time RT-PCR protocol for the detection of influenza A and B. Specificity of both assays was challenged with commercial NATrol FLU verification panel samples.

RESULTS

Both influenza A and B RT-SIBA assays were able to reliably and reproducibly detect 100 copies of viral RNA in less than 15 minutes (Fig. 2). The assays detected the clinically relevant subtypes of influenza A, namely H1N1, H1N1pdm and H3N2, as well as both Victoria and Yamagata lineages of influenza B. The results were superior to the CDC RT-PCR reference methods both in sensitivity and speed (Table 1). Ten copies of RNA was reproducibly amplified by both assays and, thus, RT-SIBA was shown to be 10-100-fold more sensitive than CDC RT-PCR depending on the influenza virus subtype. Importantly, the complete RT-SIBA test can be completed in less than 30 minutes. Further, RT-SIBA influenza A and B assays were found to be highly specific and neither of the assays cross-reacted with other common human respiratory pathogens (Table 2).

CONCLUSIONS

In addition to DNA detection, the novel RT-SIBA method extends the ability of SIBA method to be utilized for rapid and accurate diagnosis of pathogens that have RNA as genetic material. As the method is performed at a low and constant temperature, the reactions can be run using battery operated and portable devices of considerably lower complexity than those used for RT-PCR. One example of such a device is the Orion GenRead® instrument. Thus, RT-SIBA method has the potential to be used both in laboratory but also in point-of-care settings. The developed influenza A and B RT-SIBA assays showed superior performance in comparison to the CDC RT-PCR method.

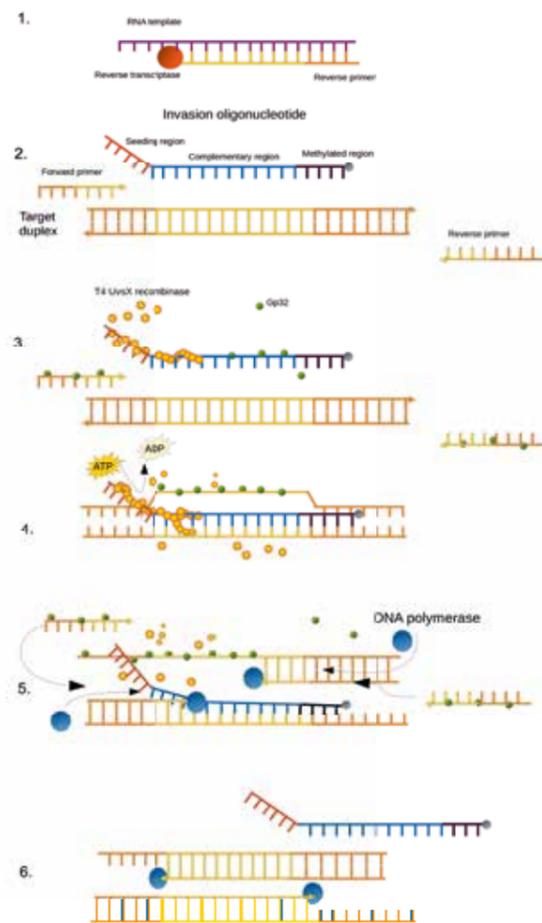


Figure 1. A mechanistic description of the RT-SIBA reaction.
 1) RNA is reverse transcribed into cDNA by RT enzyme.
 2) The SIBA reaction requires two target specific primers and an invasion oligonucleotide (IO). All single-stranded elements are coated with gp32.
 3) T4 UvsX recombinase polymerizes the IO and gp32 is displaced. Primers do not act as substrates for UvsX.
 4) IO invades the complementary region of the target duplex through the activity of UvsX. Complete separation of the target duplex follows and target specific primers bind the target.
 5-6) DNA with strand displacement activity is able to extend the dissociated target duplex from the primers. This event leads to the production of two copies of the target duplex. The recombinase mediated target duplex separation and polymerase mediated extension are the basis for exponential amplification.

Table 1. Sensitivity of the influenza A and B RT-SIBA assays, compared with equivalent CDC RT-PCR assays.

Subtype	Copies/reaction	No. of positive reactions (average time to positive result*)			
		RT-SIBA		RT-PCR	
A (H1N1)	1000	12/12 (12 min)		12/12 (54 min)	
	100	12/12 (15 min)		0/12	
	10	12/12 (20 min)		0/12	
A (H3N2)	1000	12/12 (11 min)		12/12 (50 min)	
	100	12/12 (14 min)		12/12 (52 min)	
	10	12/12 (16 min)		0/12	
A (H5N1)	1000	12/12 (10 min)		12/12 (51 min)	
	100	12/12 (12 min)		12/12 (53 min)	
	10	12/12 (15 min)		0/12	
B	1000		12/12 (10 min)		12/12 (51 min)
	100		12/12 (12 min)		12/12 (53 min)
	10		11/12 (13 min)		11/12 (56 min)

*ramp time of RT-PCR reactions not included in the calculations.

Table 2. NATrol FLU verification panel test results.

NATrol FLU Verification Panel (ZeptoMetrix, Buffalo, NY, USA) samples were used to study the ability of RT-SIBA to specifically detect influenza viral particles in the presence of a simulated clinical sample matrix. Each sample was transferred using flocked swabs into a lysis buffer (10 % Triton X-100, Sigma-Aldrich, USA) and 2 µl was then transferred to the reaction. Data is shown as time to detection in minutes and represents averages of five replicate reactions. The manufacturer did not indicate the number of viral particles per sample.

Panel Member	Strain	RT-SIBA Result, Influenza A	RT-SIBA Result, Influenza B
Influenza A H1N1	A/NewCaledonia/20/99	19.4	-
Influenza A H1N1	A/Brisbane/59/07	20.1	-
Influenza A H3N2	A/Brisbane/10/07	17.4	-
Influenza A H3N2	A/Wisconsin/67/05	18.4	-
Influenza A 2009 H1N1	Canada/6294/09	18.6	-
Influenza A 2009 H1N1	NY/02/09	19.9	-
Influenza B	B/Florida/02/06	-	14.8
Influenza B	B/Malaysia/2506/04	-	18.1
Respiratory Syncytial Virus A	NA	-	-
Respiratory Syncytial Virus B	CH93(18)-18	-	-
Rhinovirus 1A	NA	-	-
Parainfluenza virus Type 1	NA	-	-
Echovirus Type 30	NA	-	-
Coxsackievirus type A9	NA	-	-
<i>M. pneumoniae</i>	M129	-	-
<i>N. meningitidis</i> Serogroup A	NA	-	-

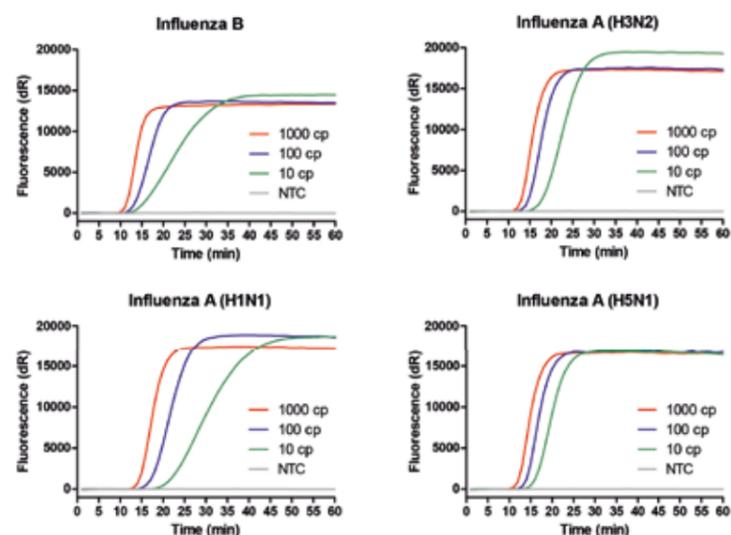


Figure 2. Sensitivity and inclusivity of influenza A and B RT-SIBA assays. Amplirun purified and quantified RNA from influenza A (H1N1, H3N2, and H5N1) and B were obtained from Vircell (Granada, Spain) and used to determine the analytical sensitivity of both Influenza A and B RT-SIBA assays. Data is plotted from one representative experiment, and the data is average of four replicate reactions. The results showed that RT-SIBA reproducibly detected and amplified as few as ten copies of influenza RNA per reaction. NTC = no template control.

REFERENCES

1. Hoser MJ, Mansukoski HK, Morrill SW, Eboigbodin KE. (2014) Strand Invasion Based Amplification (SIBA®): a novel isothermal DNA amplification technology demonstrating high specificity and sensitivity for a single molecule of target analyte. PLoS One. Nov 24;9(11):e112656



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