Reverse Transcription Strand Invasion Based Amplification (RT-SIBA) Method for rapid detection of human rhinoviruses

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BACKGROUND

Upper respiratory tract infection (URTI) which includes the common cold is the most prevalent human illness. In addition to the high incidence, the infection carries an enormous economic burden. Data extrapolated from Finland shows that the direct and indirect cost related to common cold, equals the total budget of occupational healthcare services in Finland. This infection costs approximately half a billion euros annually for the Finnish industry and trade. Human rhinoviruses (HRVs) are the predominant cause of common cold. Rhinoviruses are also linked to exacerbations of chronic pulmonary disease, asthma development, severe bronchiolitis in infants and children, and fatal pneumonia in elderly and immunocompromised adults.

As there are more than 150 rhinovirus serotypes infecting humans, rapid, sensitive and specific diagnostics methods, which discriminate all picornavirus serotypes, are needed in order to direct the treatment of respiratory infections correctly and to avoid unnecessary antibiotic prescriptions. Real-time reverse transcription polymerase chain reaction (RT-qPCR) has been developed for the diagnosis of HRVs, offering improved sensitivity over the time-consuming virus culture method. However, RT-qPCR requires thermal cyclers and skilled personnel, which consequently limit its use in field or point-of-care applications. In this study, we report the development of an isothermal nucleic acid amplification method for the detection of HRVs. The method does not require thermal cyclers or heavy instruments and is as such suitable for field or point-of-care applications.

METHODS

We previously described a novel isothermal nucleic acid amplification method called Strand Invasion Based Amplification (SIBA®) with high analytical sensitivity and specificity[1]. The method relies on the recombinase-dependent insertion of a single-stranded invasion oligonucleotide (IO) for the dissociation of the target duplex. This event allows target specific primers to bind and extend the target via the action of a DNA polymerase. The method was previously found to be useful for the rapid detection of DNA from pathogens[1]. In this study, we developed a reverse transcription SIBA (RT-SIBA) for the rapid detection of viral RNA targets. RT-SIBA assay was designed to detect the conserved sequences within the 5' untranslated region of HRV genome. The method further includes a reverse transcriptase enzyme that allows an one-step reverse transcription of RNA to cDNA and simultaneous amplification and real-time detection of cDNA with SIBA. *In vitro* transcribed RNA harboring the 5' untranslated region of HRV was used for the determination of assay analytical sensitivity. The reaction was performed at low and constant temperature (41°C) eliminating the need for the repeated cycles of heating and cooling steps.

RESULTS AND CONCLUSION

The RT-SIBA was able to detect a considerably low amount of *in vitro* transcribed RNA or virus particles of HRVs within 30 minutes. RT-SIBA was found to be a rapid, specific and sensitive method for the detection of viral RNA and has great potential as a powerful diagnostic tool in centralised and decentralised test settings.

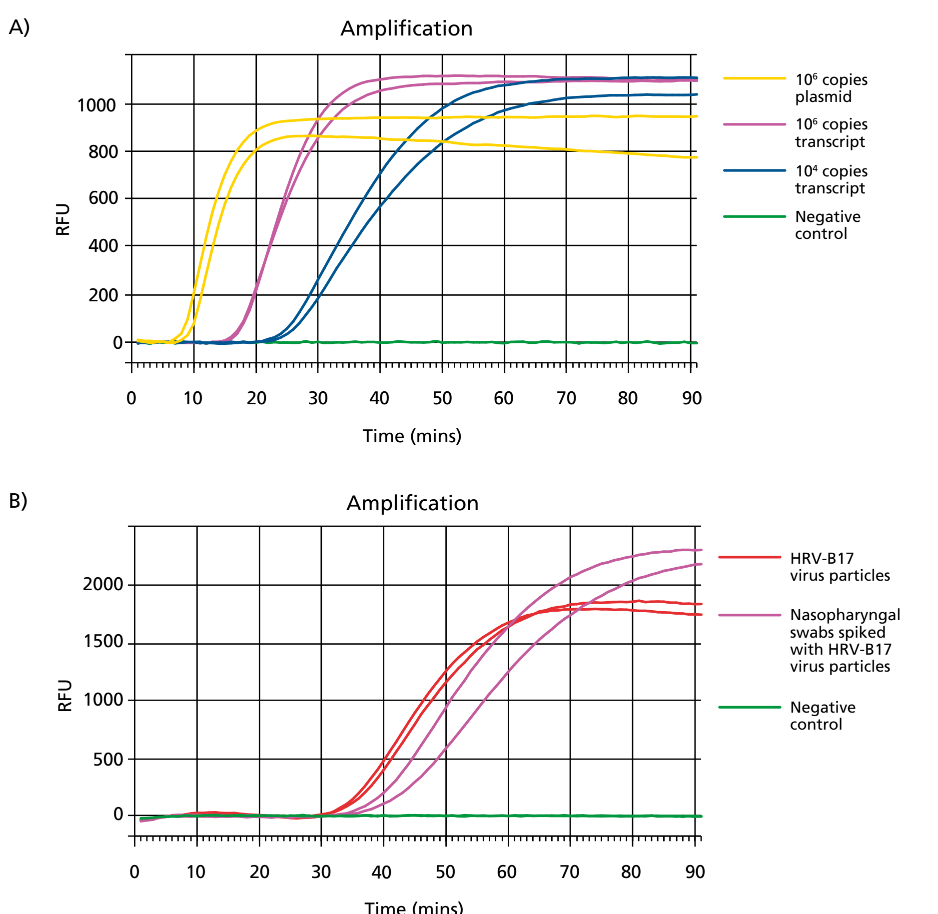

Figure 1. Amplification of HRV RNA
A) 10^4 (blue) and 10^6 (purple) copies of *in vitro* transcribed RNA or 10^6 copies of control plasmid (orange) as templates. B) HRV-B17 virus particles (red) and nasopharyngal swabs spiked with HRV-B17 virus particles (pink). The virus particles both with and without sample matrix amplified well in the test system. Samples were prepared using a simple in-house RNA extraction protocol. Samples without RNA (A) or virusparticles (B) are shown as negative controls (green). The reactions were detected with Sybr Green 1.

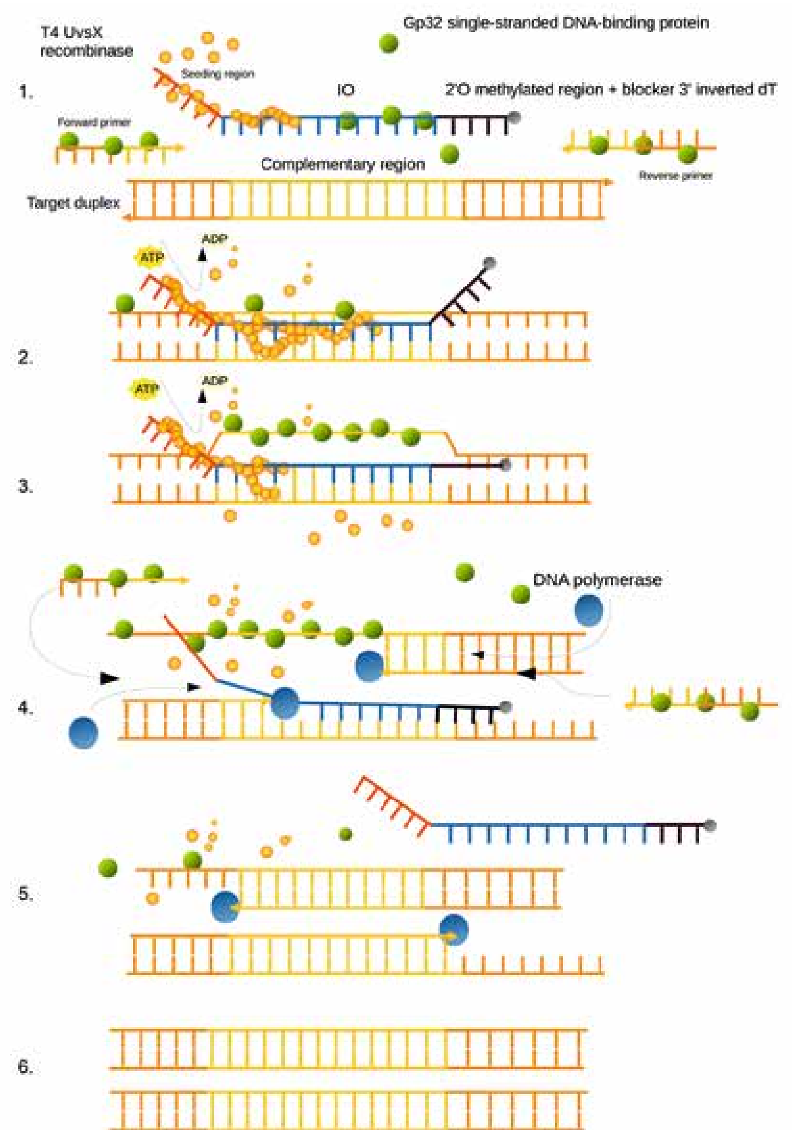

Figure 2. General description of the SIBA reaction

1) Single-stranded binding protein, Gp32 coats all single-stranded oligonucleotides (weakly coating the 2'-O-methyl RNA nucleotides). The ATPase dependent recombinase, UvsX, coats the invasion oligonucleotide (IO) displacing gp32. UvsX only weakly coats the primers since they are too short to acts as a recombinase substrate.
2) The UvsX-IO complex invades the complementary region of the target duplex. This induces a partial separation of the target duplex.
3) Depolymerisation of UvsX, allows the 2'-O-methyl RNA region of the IO to branch migrate onto the downstream region of the target duplex. This results in the complete separation for the target duplex.
4) Target specific primers are now able to bind the target and are extended by the action of a strand displacement DNA polymerase.
5) The IO is displaced during extension of the target DNA, and is re-used for subsequent round of strand invasion events.
6) Two copies of the target duplex are produced. The recombinase mediated target duplex separation and the polymerase mediated extension is the basis for the exponential amplification.

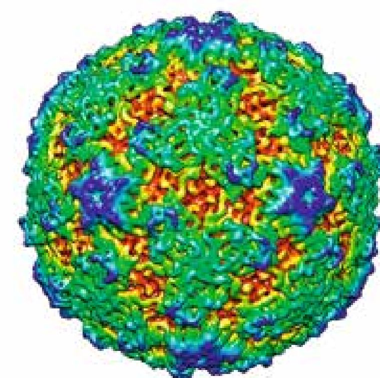

Figure 3. CryoEM reconstruction of Human Rhinovirus RV-A2 (Courtesy of Dr. Dieter Blaas, Medical University of Vienna, Austria).

REFERENCES

1. Hoser MJ, Mansukoski HK, Morrical SW, Eboigbodin KE: 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 2014, 9(11):e112656.

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