Rapid Identification of Respiratory Infectious Disease Viruses Using Stable High-Frequency Mutation Sites

Jianxin Lyu[1], Linjie Chen[1], Yu Fu[1], Xiaobai He[1], Fei Kong[1], Quan fang[1], Yanxin Liu[1], Yan Zhang[1], and Liang Chen[1]

[1]Hangzhou Medical College

July 19, 2023

Abstract

Background: Respiratory infectious viruses, such as the novel coronavirus SARS-CoV-2, are highly transmissible and undergo rapid genetic evolution, which leads to the emergence of multiple subtypes with diverse mutation patterns. However, detecting and differentiating between these subtypes present significant challenges in the field of respiratory virus surveillance. Methods: To address these challenges, we developed a novel detection approach that integrates amplification refractory mutation system PCR (ARMS-PCR) with molecular beacon probes. The ARMS-PCR primers were designed to selectively amplify specific subtypes by targeting adjacent mutation sites, while the molecular beacon probes allowed for further discrimination of the amplified products. This combined approach effectively addressed the issues of non-specific binding and improved detection accuracy. Results: Our method demonstrated high specificity and sensitivity in the identification and differentiation of respiratory virus subtypes. Using real-time fluorescence PCR, we achieved a detection limit of approximately 106 copies/mL. Moreover, through the direct analysis of fluorescence signals, we further enhanced the sensitivity to a detection limit of 104 copies/mL. This robust and accurate detection approach is capable of identifying and differentiating between respiratory virus subtypes, including those with complex mutation patterns. Conclusions: The integration of ARMS-PCR and molecular beacon probes is a reliable and efficient solution for the rapid and precise monitoring of evolving respiratory infectious diseases, and it has the potential to facilitate early diagnosis and effective control measures. Further research is needed to expand the application of this detection method to other respiratory viruses and optimize its workflow for clinical and public health settings.

Hosted file

`main.docx` available at https://authorea.com/users/640912/articles/655540-rapid-identification-of-respiratory-infectious-disease-viruses-using-stable-high-frequency-mutation-sites

Hosted file

`Figure1.pptx` available at https://authorea.com/users/640912/articles/655540-rapid-identification-of-respiratory-infectious-disease-viruses-using-stable-high-frequency-mutation-sites

Hosted file

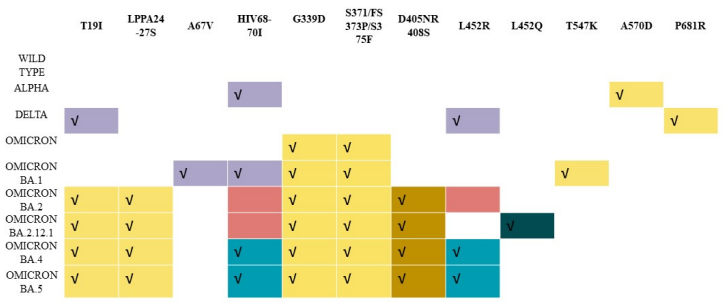
`Figure2.pptx` available at https://authorea.com/users/640912/articles/655540-rapid-identification-of-respiratory-infectious-disease-viruses-using-stable-high-frequency-mutation-sites

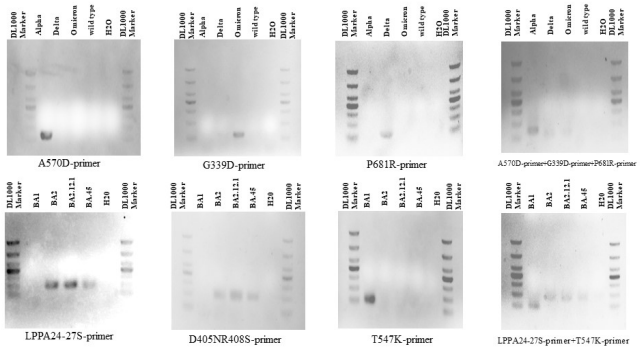
Hosted file

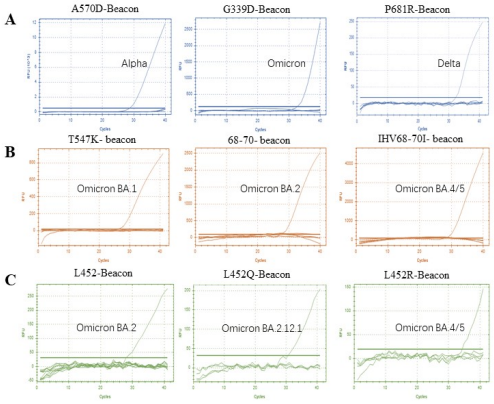
Figure3.pptx available at https://authorea.com/users/640912/articles/655540-rapid-identification-of-respiratory-infectious-disease-viruses-using-stable-high-frequency-mutation-sites

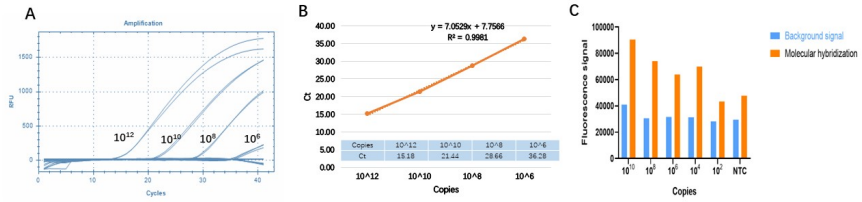
Hosted file

Fiture 4.pptx available at https://authorea.com/users/640912/articles/655540-rapid-identification-of-respiratory-infectious-disease-viruses-using-stable-high-frequency-mutation-sites









variant subtype	Sequence information	SNP	Forward primer	Reverse primer	Beacon
WILD TYPE	NC_045512.2				
Delta	EPI_ISL_12089695.1	P681R	ACTCAGACTAATTCTTG	ATAGAGTTATTAGAGTAAGCA	**CCGGCT**TAGCTAGTCAATCCATCATTGCC**AGCCGG**
Alpha	EPI_ISL_12074954.1	A570D	CAACAATTTGGCAGAGACATTCA	CAGCAACCTGGTTAGAAGTATT	**CGCACC**TGATGCTGTCCGTGATCCACAGACACTTGAGATTCTTGACATTACAC**GGTGCG**
Omicron		G339D S371L/S373P/S375F	ACTTGTGCCCTTTTAA	AGTGAAAAATGGTGCGA	**CGCACC**TTAACGCCACCAGATTTGCATCTGTTTATGC**GGTGCG** R346K-BA.1.1 **CGCACC**TTAACGCCACCAAATTTGCATCTGTTTATGC**GGTGCG**
BA.1	EPI_ISL_12832808.1	T547K	CAACTTCAATGGTTTAGA	CATCAGTAGTGTCAGCAA	**CGCGCC**TGTTAGACTCAGTAAGAACACCT**GGCGCG**
		T19LLPPA24-27S	T19LLPPA24-27S-F: TATAACCAGAACTCAC TC	T19LLPPA24-27S-R: AGGGTTATCAAACCTCTTA	68-70-BA.2/BA.2.12.1: **CGCTCG**TCCCAGAGACATGTATAGCATGG**CGAGCG**
BA.2	EPI_ISL_12081490.1	D405NR408S	D405NR408S-F TAGAGGTAATGAAGTCTGC	D405NR408S-R: TATCTCTCTCAAAAGGTT	L452-BA.2: **CGACGCT**GGTAATTATAATTACCTGTATAGATTGTTTAG**AGCGTCG**
BA.2.12.1	EPI_ISL_12690472.1	D405NR408S L452Q	D405NR408S-F TAGAGGTAATGAAGTCTGC	D405NR408S-R: TATCTCTCTCAAAAGGTT	**CCTCGCT**GGTAATTATAATTACCAGTATAGATTGTTTA**GAGCGACG**
BA.4/5	BA.4: EPI_ISL_12660507.1	T19LLPPA24-27S IHV68-70I	T19LLPPA24-27S-F: TATAACCAGAACTCAC TC	T19LLPPA24-27S-R: AGGGTTATCAAACCTCTTA	IHV68-70I-BA.4/5 **CGCTCG**TGGTCCCAGAGATAGCATGGAAC**CGAGCG** L452R-BA.4/5:
	BA.5: EPI_ISL_12659704.1	D405NR408S L452R	D405NR408S-F TAGAGGTAATGAAGTCTGC	D405NR408S-R: TATCTCTCTCAAAAGGTT	**CGTCGAG**GGTAATTATAATTACCGGTATAGATTGTTTA**GCTCGACG**

Hosted file

Table1.pptx available at https://authorea.com/users/640912/articles/655540-rapid-identification-of-respiratory-infectious-disease-viruses-using-stable-high-frequency-mutation-sites