

Evaluation of respiratory viruses multiplex assay using RT-qPCR

Monica Arruda¹, Pedro Henrique Cardoso¹, Marisa Ribeiro¹, Alexandre Calazans¹, Fernando do Couto Motta², Marilda Agudo Mendonça Teixeira de Siqueira², Patrícia Alvarez¹
1- LAMOL / 2- LVRE

Introduction

Viruses like Influenza A(INFA), Influenza B(INFB), SARSCoV-2(SC2), Respiratory Syncytial Virus (RSV), Human Metapneumovirus (HMPV), Adenovirus (ADV) and Rhinovirus (HRV) are etiological agents of acute upper respiratory diseases that can affect the bronchi and lungs and represent an important cause of pneumonia in children and adults. Some of these viruses can evolve and spread quickly as in the H1N1 2009 and COVID-19 global pandemics. With the development of new diagnostic tests, the wieldy of more accurately differentiating these viruses is fundamental for clinical management with more efficient treatments to control the impact of respiratory tract infections. The aim of this study was to evaluate the prevalence of respiratory viruses (RV) in Brazil, using multiplex real-time PCR methodology to identify INFA, INFB, SC2, HMPV, RSV, ADV, HRV through the assay VR1/VR2, developed at Bio-Manguinhos/Fiocruz as an epidemiological surveillance study of the main and most prevalent circulating respiratory viruses.

Methods

The VR1/VR2 Bio-Manguinhos multiplex molecular assay is composed of 2 modules, able to discriminate VR and the human constitutive gene RNase P (RP) as an internal reaction control, with VR1 identifying INFA, INFB, SC2, HMPV and VR2 identifies RSV, ADV, HRV.

Results

This trial was distributed in the multicenter study with the LACENs: TO, SE, BA, MG, RN, GO, SC and RS, in June 2023 (Figure 1). The study received 2667 results, following the VR1/VR2 amplification profile of the samples (Figure 2). Of these samples we identified 1034 (38.77%) positive samples for VR, with 33 (1.24%) positive results in samples from INFA, INFB 22 (0.82%), SC2 154 (5.77%), HMPV 98(3.67%) and ADV 45(1.69%), RSV 180(6.75%) and HRV 502(18.82%). In the distribution by LACEN distribution, we identified a frequency of positive samples among all viruses of 27.03% in TO, 24.51% in SE, 58.32% in BA, 29.44% in MG, 29.03% in RN, 43.01% in GO, 54.65% in SC and 43.48% in RS. The highest prevalence among the viruses was HRV in all states.

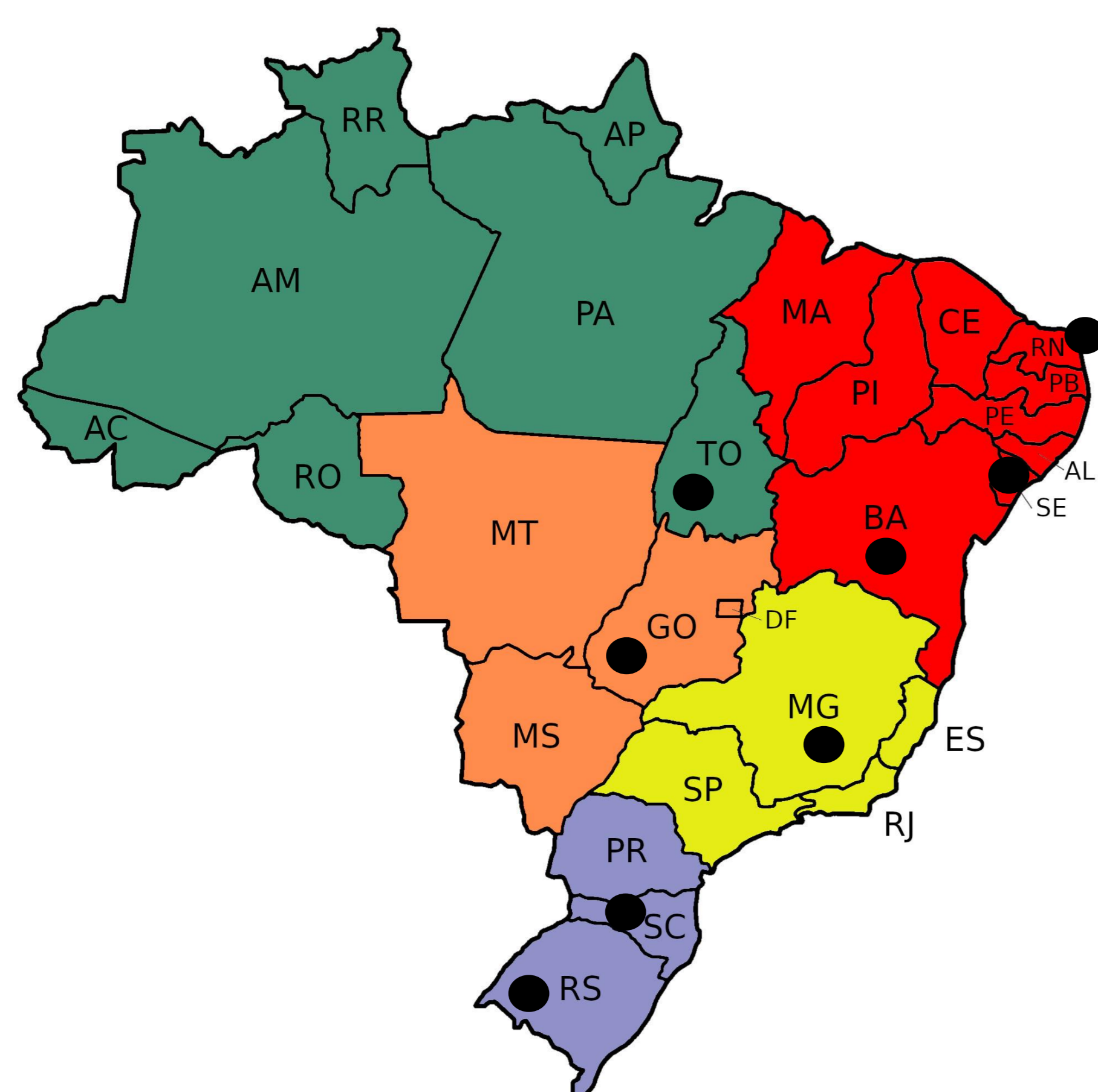


Figure 1 - Multicenter study with the LACENs: TO, SE, BA, MG, RN, GO, SC and RS, in June 2023.

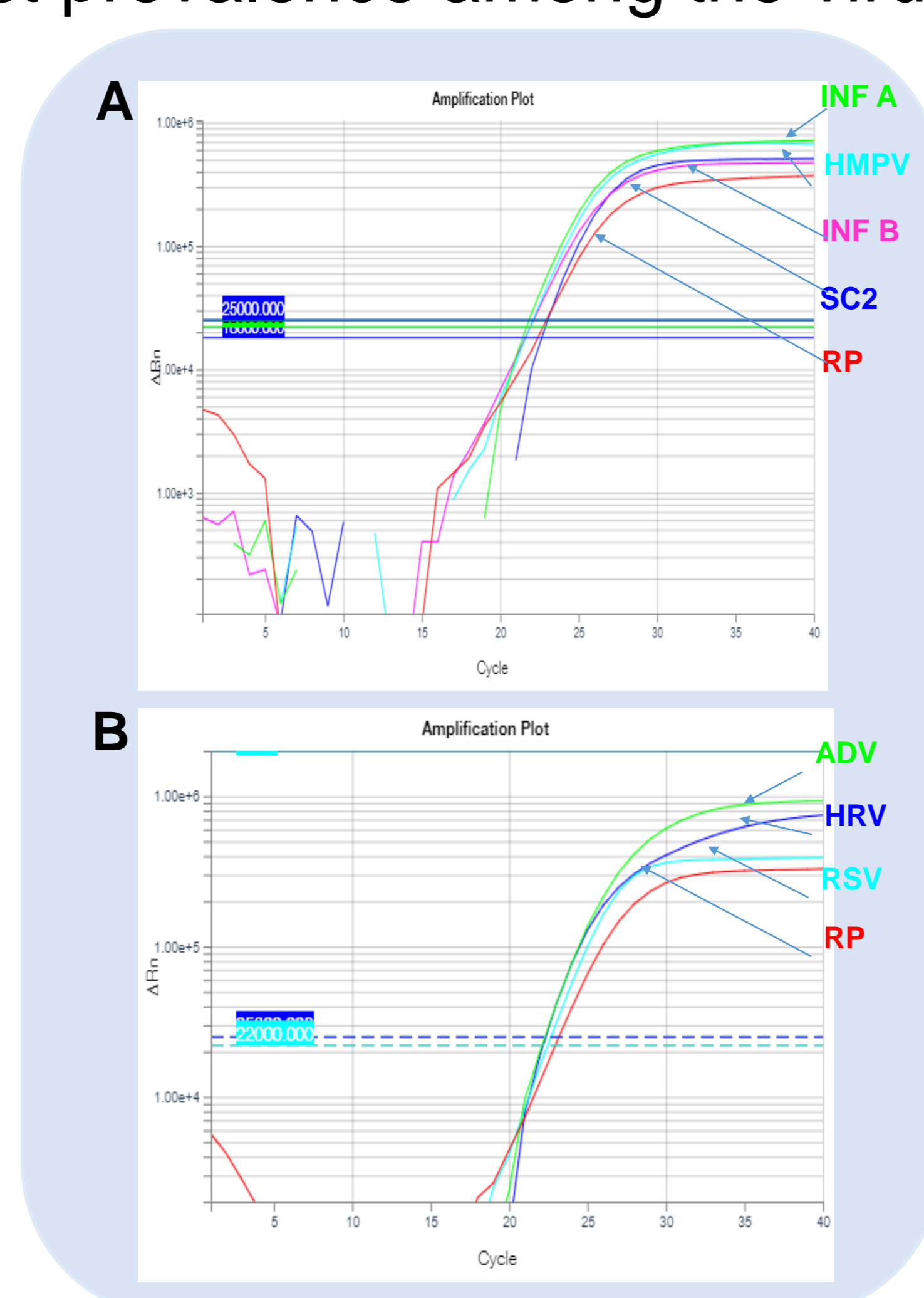


Figure 2 – Amplification profile of samples tested for VR1 (A) and VR2 (B)

SARS	No. of samples detected	Frequency (%)
HRV	502	18,82
RSV	180	6,75
SC2	154	5,77
HMPV	98	3,67
ADV	45	1,69
INFA	33	1,24
INFB	22	0,82
Total samples detected	1034	38,77

Figure 3 - Percentage of samples detectable for SARS by VR1/VR2

Conclusion

These results demonstrate the efficiency in identifying the main VR, and the importance of identifying and monitoring these viruses in Brazil, mainly using a multiplex product that can identify the 7 viruses in just 2 reactions in conjunction with the internal control. Furthermore, this molecular strategy can function work as an epidemiological surveillance.