Case report: 90 year old lady infected with two CoVID-19 VOCs: 20I/501Y.V1 and 20H/501Y.V2

Anne Vankeerberghen, Astrid Holderbeke, An Boel, Hans De Beenhouwer, Kristien Van Vaerenbergh and Lien Cattoir
Laboratory of Clinical Microbiology, OLVZ Aalst, Belgium

INTRODUCTION

In March 2021, a 90 year old lady was admitted to our hospital because of recurrent falling. Screening for SARS-CoV-2 was positive. She had no important medical history. She did not reside in a collectivity, she lived alone and received nursing care at home. Initially, there were no signs of respiratory distress and the patient had good oxygen saturations. However, during her stay, she developed rapidly worsening respiratory symptoms. The patient died after five days.

METHODS

1. Extraction of SARS-CoV-2 RNA from respiratory samples
Seegene STARMag 96x4 Viral DNA/RNA 200 C Kit
Hamilton STARlet extraction- and pipetting station

2. Real time PCR for detection of SARS-CoV-2
TaqMan PCR: Primers and probes according to CDC, N1 PCR
TaqMan Fast Virus 1-Step master mix
Quantstudio 7 flex real time PCR system
Fastfinder data analysis software

2. Real time PCR mutation analysis for determination of Variant of Concern (VOC)
Primers and MGB probes designed with primer express version 3 (Applied biosystems)
Multiplex wildtype PCR for N501Y, HV69-70del and A701V
Multiplex mutation PCR for N501Y, HV69-70del and A701V with the mutation located in the forward or reverse primer
Quantstudio 7 flex
Algorithm:

	HV69-70Del	N501Y	A701V
Alpha (20I/501Y.V1)	MUT	MUT	WT
Beta (20H/201Y.V2)	WT	MUT	MUT

3. Sanger sequencing of SARS-CoV-2 S-gene
Primers designed with primer 3 Plus
Sequencing on GenomeLab Genetic Analytic System (Analis)

4. Whole genome sequencing
Artic pipeline
MinION (Oxford Nanopore)
Nextclade and Pangolin

RESULTS

1. **SARS-COV-2 DETECTION:** sample upon admission in our hospital: strongly positive: Ct 18,98. Sample obtained 3 days before admission: Ct 17,43.

2. **VOC ANALYSIS USING REAL TIME PCR**

HV69-70Del wildtype and mutant both present (both samples)
N501Y mutant present (both samples)
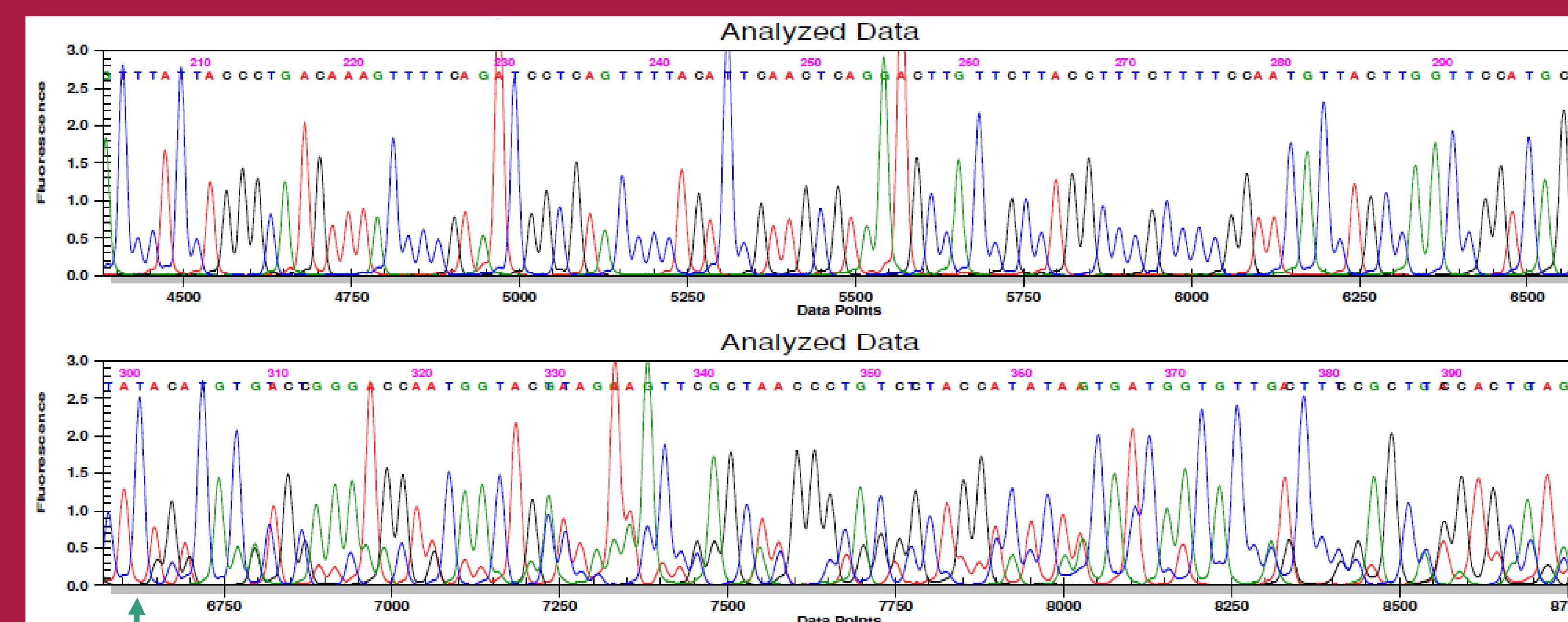A701V wildtype and mutant both present (both samples)

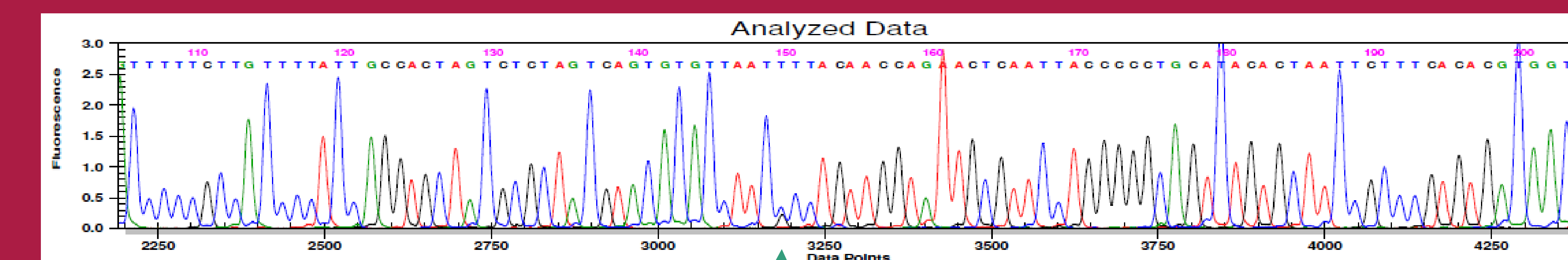→ COINFECTION WITH 20I/501Y.V1 (ALPHA) AND 20H/201Y.V2 (BETA) VARIANT
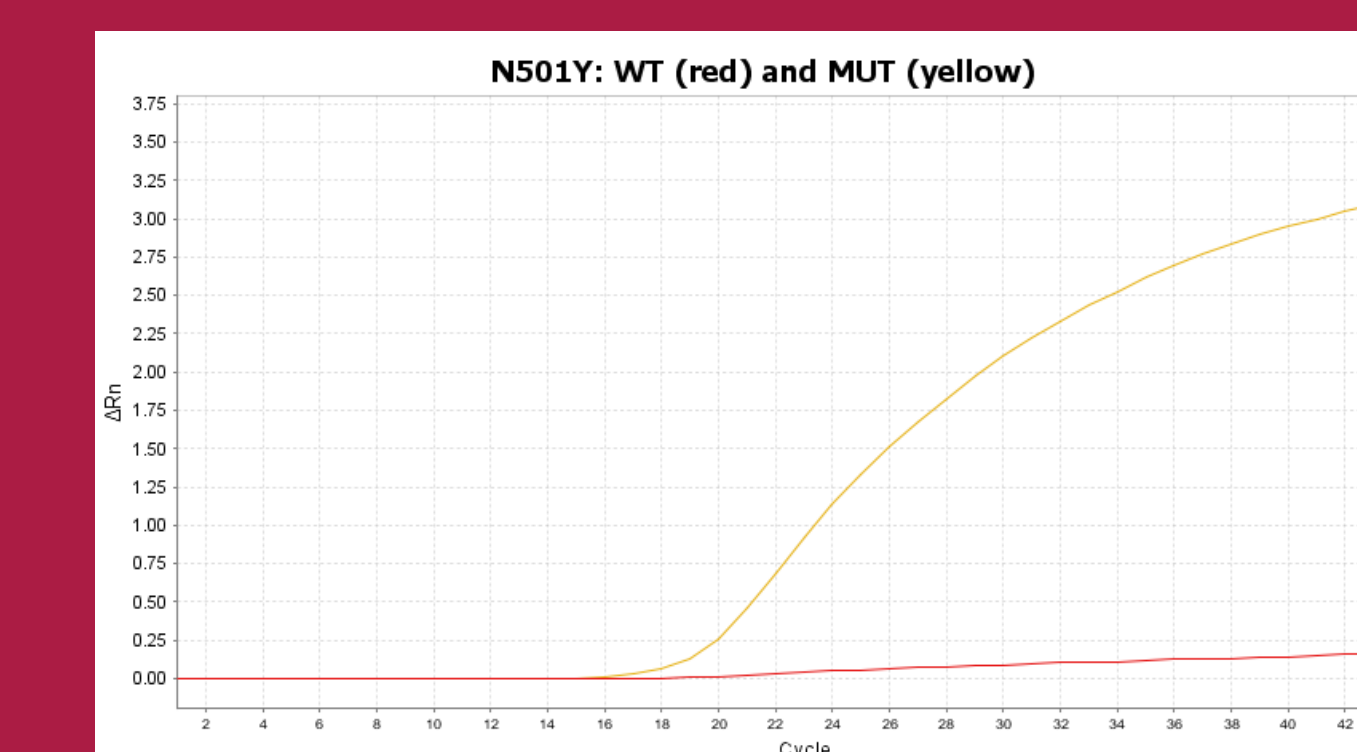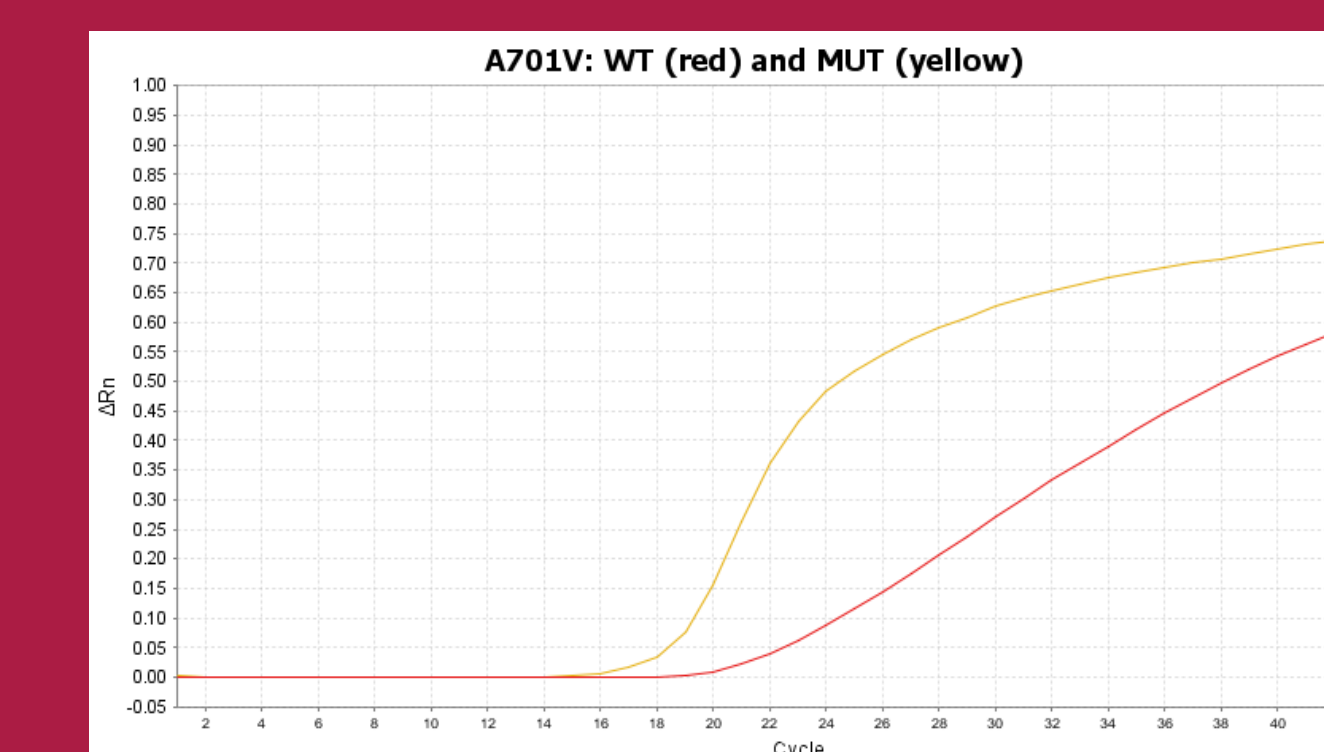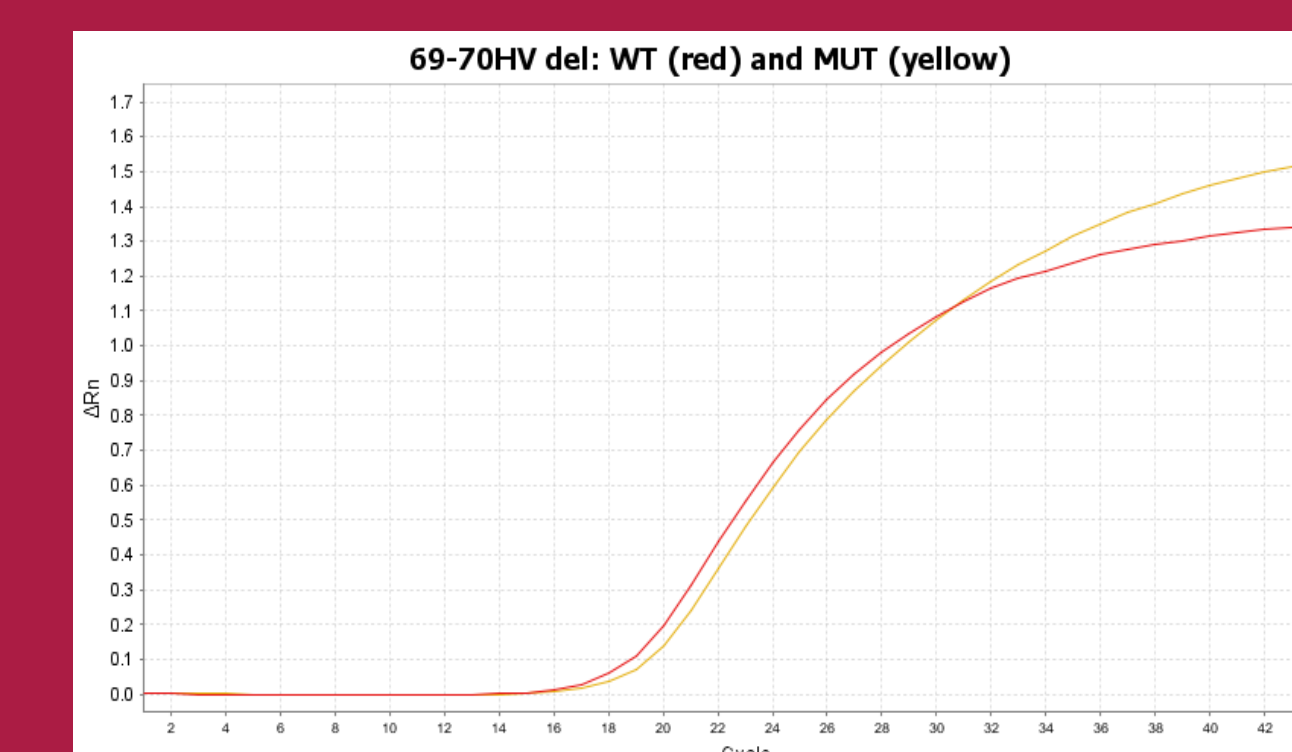
3. **SANGER SEQUENCING S-GENE OF SARS-COV-2**

Wildtype and mutant sequence present for L18F, HV69-70Del, Y144Del (not shown) and E484K. Only mutant sequence for N501Y.
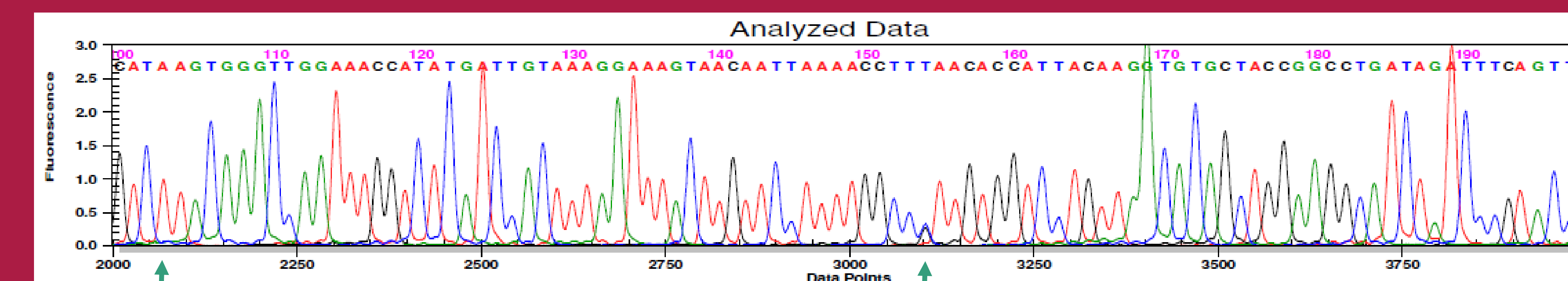
→ COINFECTION WITH 20I/501Y.V1 (ALPHA) AND 20H/201Y.V2 (BETA) VARIANT

HV69-70Del (21765-21770del); forward sequence

L18F (C21614T); forward sequence

N501Y (A23063T); reverse sequence

E484K (G23012A); reverse sequence

4. **MINION WHOLE GENOME SEQUENCING OF SARS-COV-2**

Nextclade / Pangolin analysis: 20H/501Y.V2 (beta) variant of SARS-CoV-2 (both samples)
Analysis of BAM-files (IGV): on average 70% beta variant and 30% alpha variant

→ COINFECTION WITH 20I/501Y.V1 (ALPHA) AND 20H/201Y.V2 (BETA) VARIANT CAN ONLY BE DETECTED WHEN BAM-FILE ANALYSIS IS PERFORMED

CONCLUSIONS

Routine VOC PCR analysis of all our positive samples identified a double infection with two SARS-CoV-2 VOC's. More widespread implementation of VOC PCR analysis of positive samples would probably identify more mixed infection and could lead to a better insight for their effect on illness and treatment.

ACKNOWLEDGEMENTS

CONTACT INFORMATION

Anne.vankeerberghen@olv-aalst.be