V4 Young Researcher Award

Mgr. Kristína Boršová, PhD.

My PhD. Study

Initially focused on:

- Molecular detection and genetic characterization of hantaviruses in Slovakia

Interruption by pandemic COVID-19:

- Routine diagnostic of SARS-CoV-2
- Development and clinical validation of a new RT-qPCR diagnostic kits
- Next generation sequencing of SARS-CoV-2 variants

Diagnostics of COVID-19

- <u>Laboratory confirmation</u>

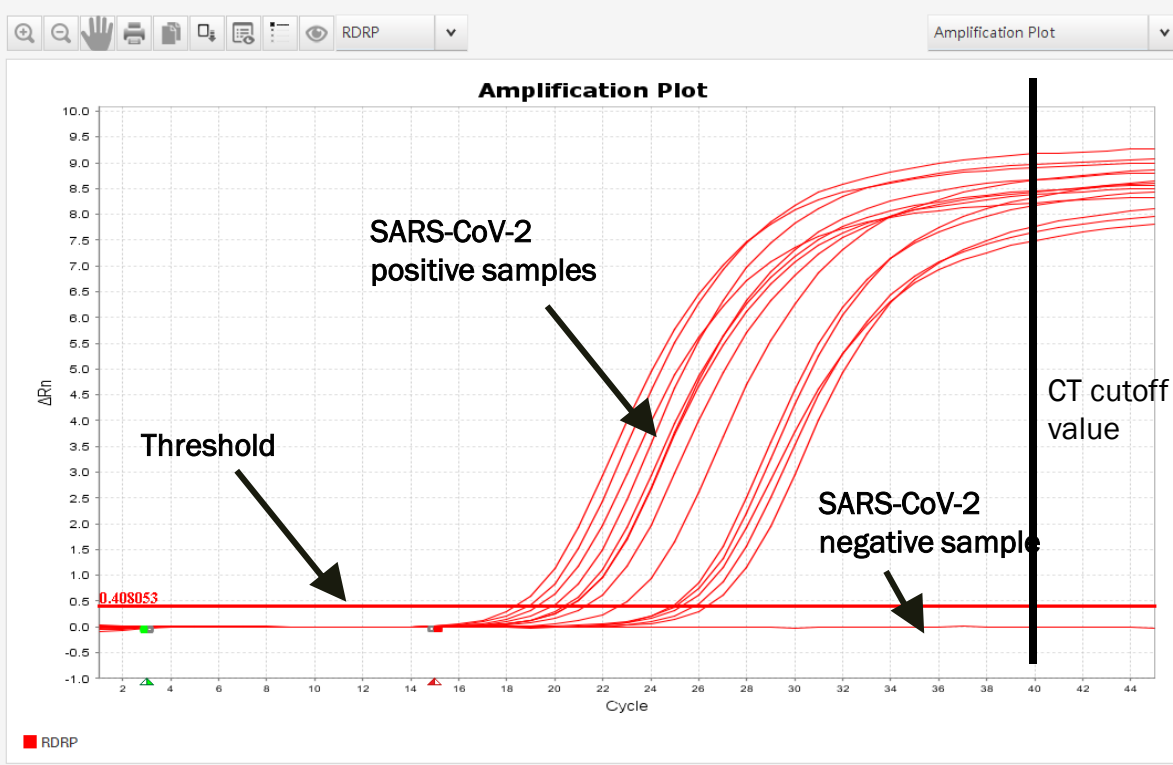
  - **RT-qPCR** (gold standard)

  - **antigen tests**

RT-qPCR - Detection the presence of one or more viral genes in a biological specimen.

- RdRp gene (RNA depedent RNA polymerase)
- E gene (Envelope gene)

Ct value - is defined as the number of cycles required for the fluorescent signal to cross the threshold. Ct levels are inversely proportional to the amount of target nucleic acid in the sample.



Clinical validation of new tests

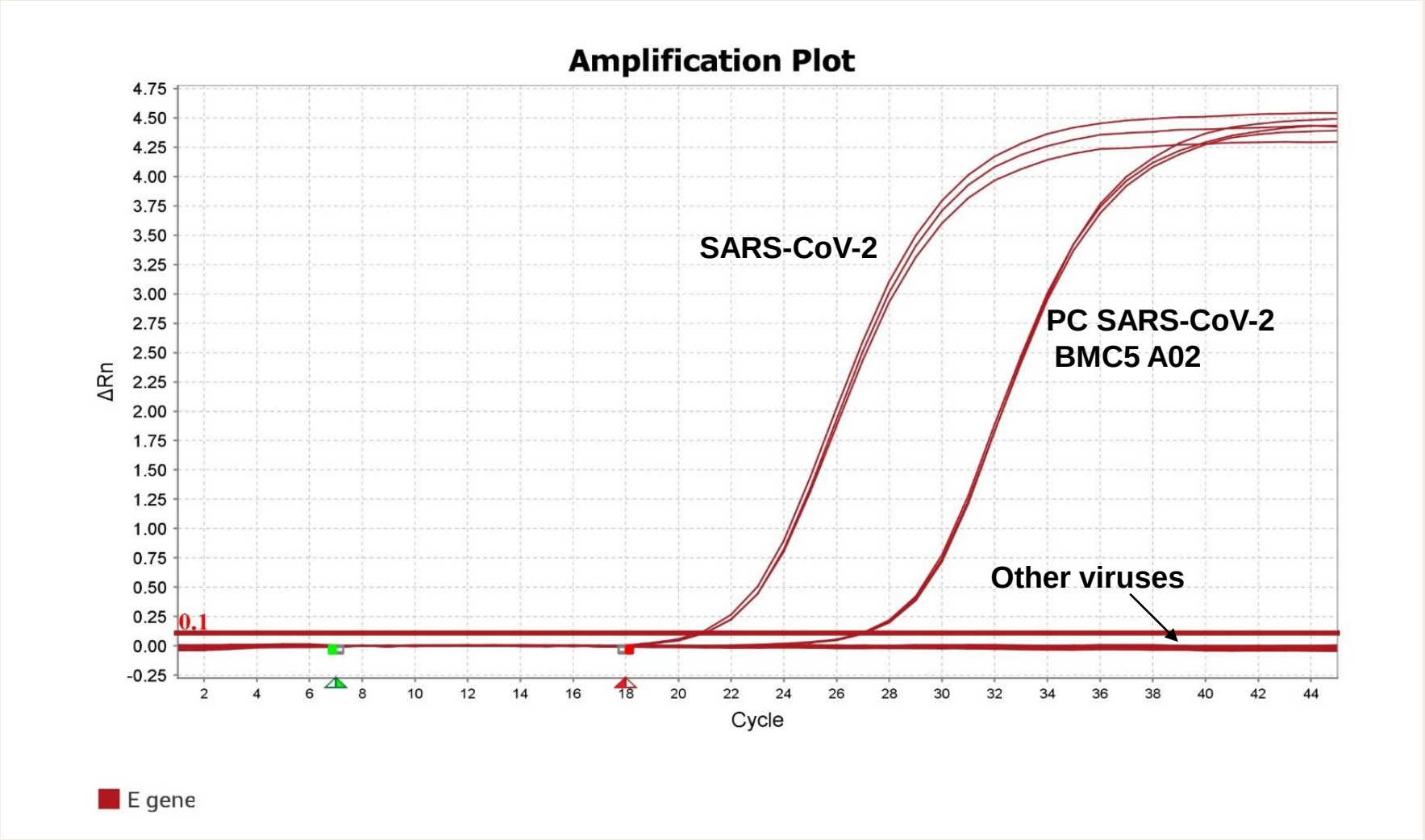
Collaboration: Private biotechnology company- Multiplex DX (development of tests)

Biomedical research center SAS (optimalization and clinical validation)

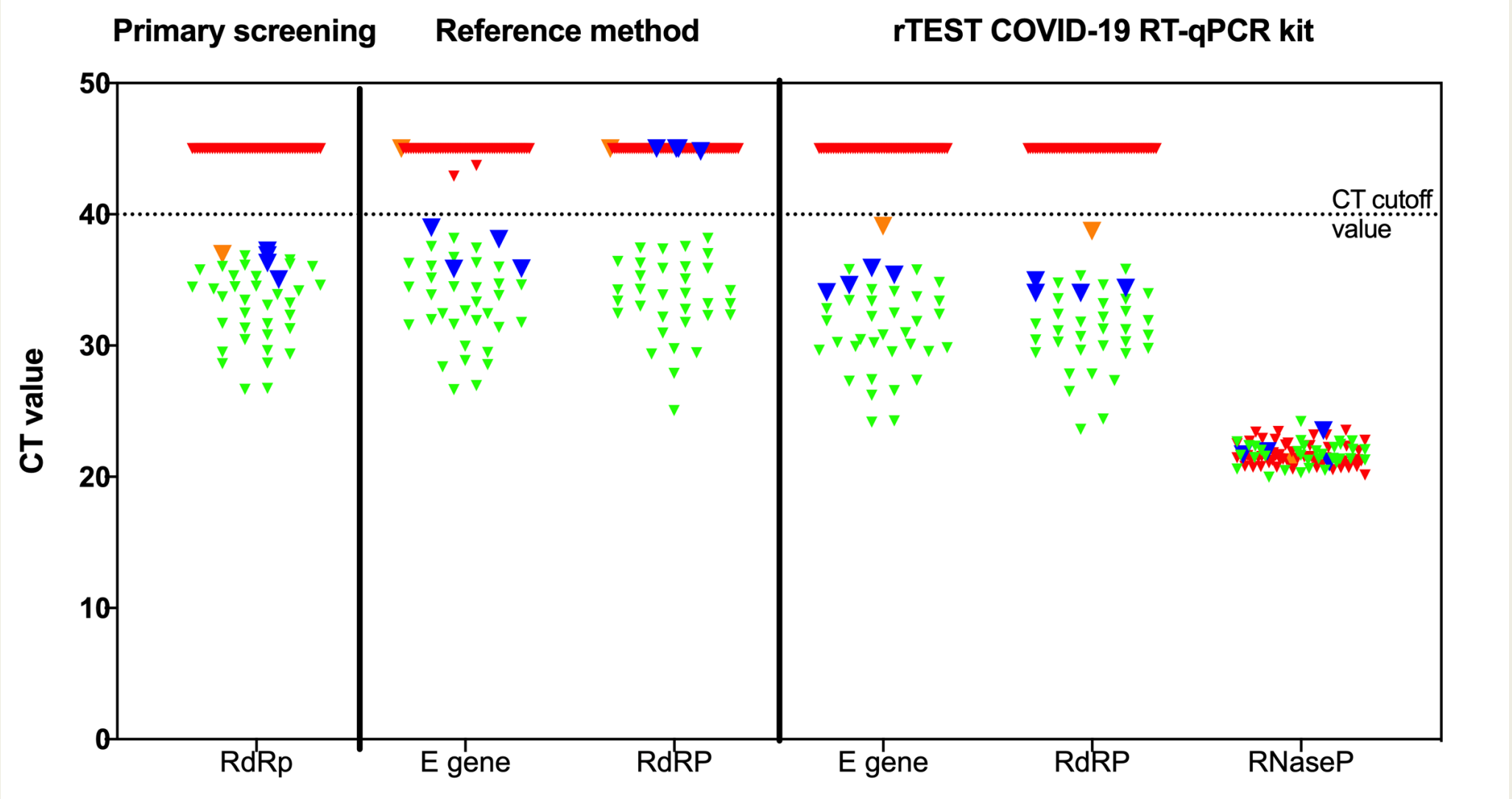
Public health authorities of the Slovak Republic (samples source)

Specificity test example

Other viruses:
HCoV-229E
HCoV-OC43
HCoV-NI63
SARS-CoV
Influenza A H1N1
Influenza A H3N2
Influenza A H5N1
Influenza B
Human parainfluenza 1
Human rhinovirus B14
RSV subtype A



Demonstration of clinical validation



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microbial biotechnology

Open Access

Sequential development of several RT-qPCR tests using LNA nucleotides and dual probe technology to differentiate SARS-CoV-2 from influenza A and B

Monika Radvánszka,[1,2,†] Evan D. Paul,[1,2,†] Roman Hajdu,[1,2,3,†] Kristína Boršová,[4] Viera Kováčová,[1,2,5] Piotr Putaj,[1,2] Stanislava Bírová,[1,2] Ivana Čirková,[1,2] Martin Čarnecký,[1,2] Katarína Buranovská,[1,2] Adrián Szobi,[1,2] Nina Vojtaššáková,[1,2] Diana Drobná,[1,2] Viktória Čabanová,[4] Monika Sláviková,[4] Martina Ličková,[4] Veronika Vaňová,[4] Sabína Fumačová Havlíková,[4] Ľubomíra Lukáčiková,[4] Ivana Kajanová,[4] Juraj Koči,[4] Diana Rusňáková,[6,7] Tatiana Sedláčková,[6,8] Klaas E. A. Max,[9] Thomas Tuschl,[9] Tomáš Szemes,[6,7,8] Boris Klempa[4,**] and Pavol Čekan[1,2,*]

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Summary

Sensitive and accurate RT-qPCR tests are the primary diagnostic tools to identify SARS-CoV-2-infected patients. While many SARS-CoV-2 RT-qPCR tests are available, there are significant differences in test sensitivity, workflow (e.g. hands-on-time), gene targets and other functionalities that users must consider. Several publicly available protocols shared by reference labs and public health authorities provide useful tools for SARS-CoV-2 diagnosis, but many have shortcomings related to sensitivity and laborious workflows. Here, we describe a series of SARS-CoV-2 RT-qPCR tests that are originally based on the protocol targeting regions of the RNA-dependent RNA polymerase (RdRp) and envelope (E) coding genes developed by the Charité Berlin. We redesigned the primers/probes, utilized locked nucleic acid nucleotides, incorporated dual probe technology and conducted extensive optimizations of reaction conditions to enhance the sensitivity and specificity of these tests. By incorporating an RNase P internal control and developing multiplexed assays for distinguishing SARS-CoV-2 and influenza A and B, we streamlined the workflow to provide quicker results and reduced consumable costs. Some of these tests use modified enzymes enabling the formulation of a room temperature-stable master mix and lyophilized positive control, thus increasing the functionality of the test and eliminating cold chain shipping and storage. Moreover, a rapid, RNA extraction-free version enables high sensitivity detection of SARS-CoV-2 in about an hour using minimally invasive, self-collected gargle samples. These RT-qPCR assays can easily be implemented in any diagnostic laboratory and can provide a

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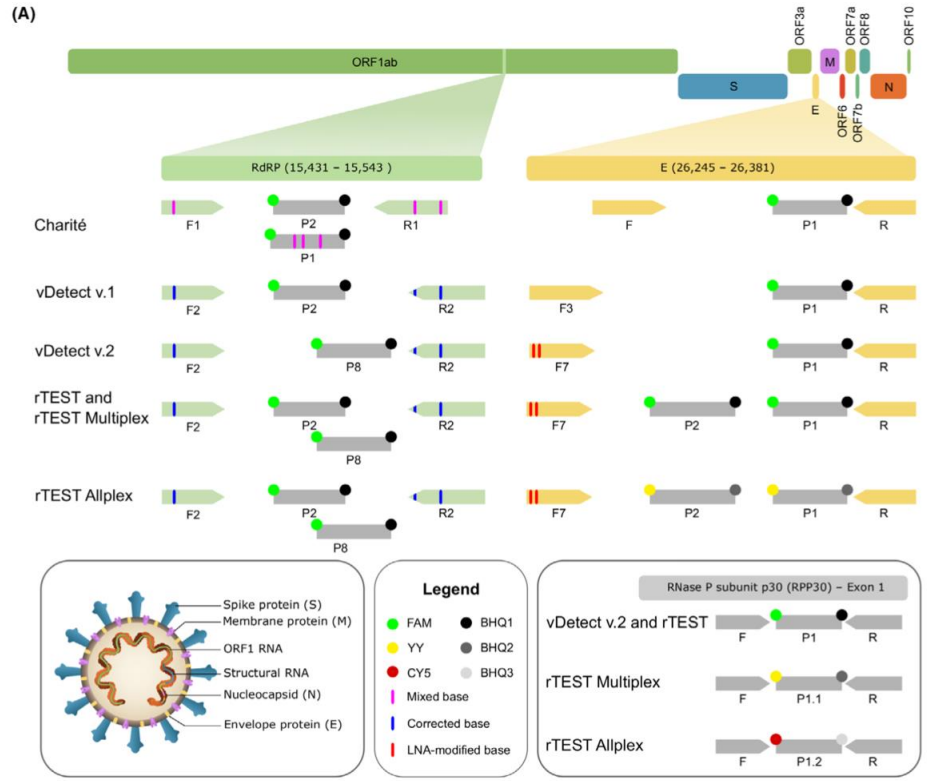


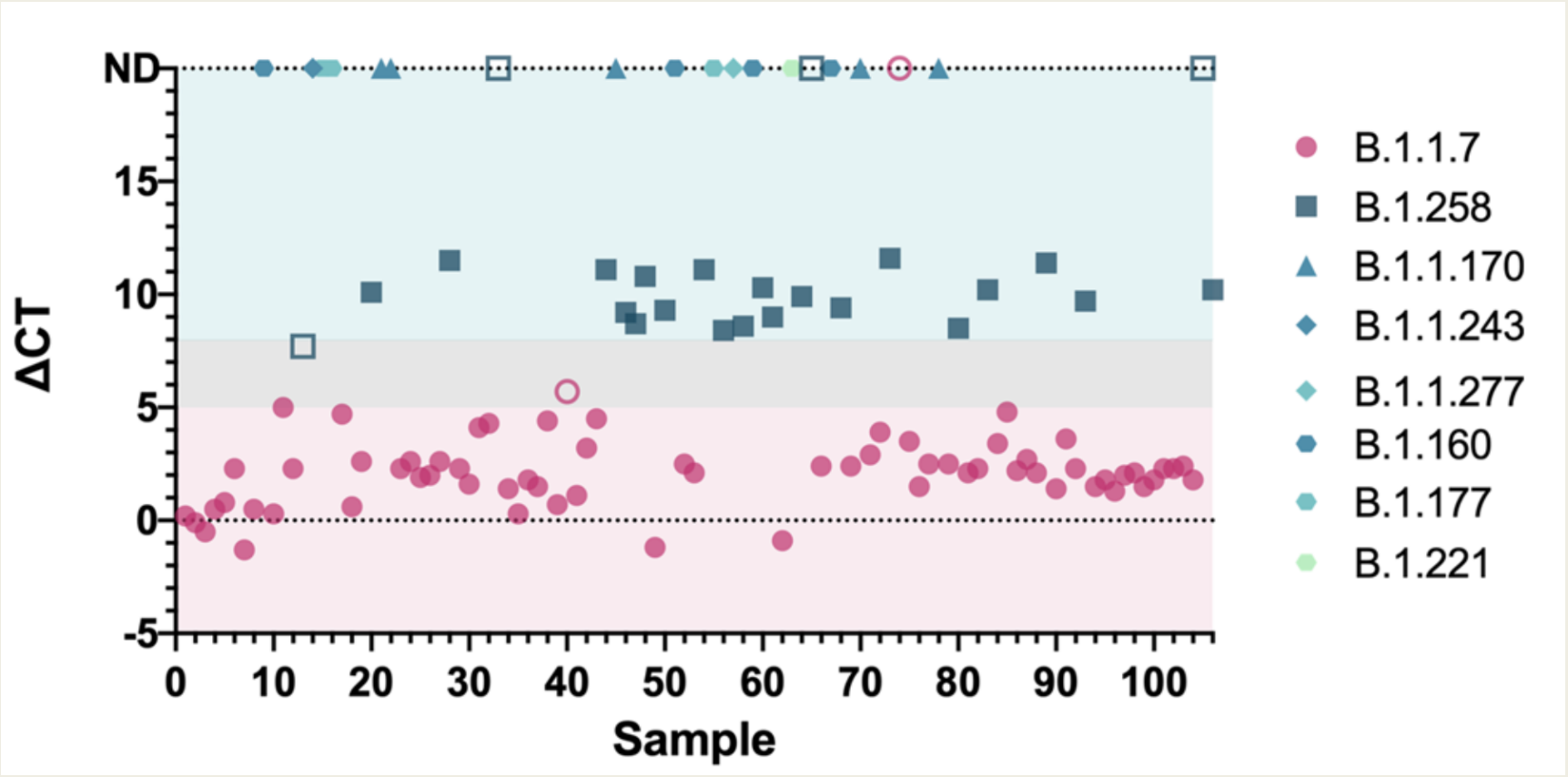
Schematic illustrating SARS-CoV-2 genome and regions targeted by RT-qPCR primers and probes. A. Schematic overview portrays the SARS-CoV-2 genome with RdRP and E gene regions magnified to show the locations of primers and probes.

rTEST COVID-19 qPCR B.1.1.7 kit

B.1.1.7 (Alpha variant)- ΔH69/ΔV70 a ΔY144

100 % sensitivity for S gene and B.1.1.7 variant

83,3 % effectiveness in identification B.1.258



Overview of ΔCt values for each sample in the clinical validation.

scientific reports

OPEN

Surveillance of SARS-CoV-2 lineage B.1.1.7 in Slovakia using a novel, multiplexed RT-qPCR assay

Kristína Boršová[3,4,22], Evan D. Paul[1,2,22], Viera Kováčová[1,2,22], Monika Radvánszka[1,2], Roman Hajdu[1,2,21], Viktória Čabanová[3], Monika Sláviková[3], Martina Líčková[3], Ľubomíra Lukáčiková[3], Andrej Belák[5,6], Lucia Roussier[6], Michaela Kostičová[6,7], Anna Líšková[8], Lucia Maďarová[9], Mária Štefkovičová[10,11], Lenka Reizigová[10,12], Elena Nováková[13], Peter Sabaka[14], Alena Koščálová[14,15], Broňa Brejová[16], Edita Staroňová[17], Matej Mišík[18], Tomáš Vinař[19], Jozef Nosek[20], Pavol Čekan[1,2]✉ & Boris Klempa[3]✉

The emergence of a novel SARS-CoV-2 B.1.1.7 variant sparked global alarm due to increased transmissibility, mortality, and uncertainty about vaccine efficacy, thus accelerating efforts to detect and track the variant. Current approaches to detect B.1.1.7 include sequencing and RT-qPCR tests containing a target assay that fails or results in reduced sensitivity towards the B.1.1.7 variant. Since many countries lack genomic surveillance programs and failed assays detect unrelated variants containing similar mutations as B.1.1.7, we used allele-specific PCR, and judicious placement of LNA-modified nucleotides to develop an RT-qPCR test that accurately and rapidly differentiates B.1.1.7 from other SARS-CoV-2 variants. We validated the test on 106 clinical samples with lineage status confirmed by sequencing and conducted a country-wide surveillance study of B.1.1.7 prevalence in Slovakia. Our multiplexed RT-qPCR test showed 97% clinical sensitivity and retesting 6,886 SARS-CoV-2 positive samples obtained during three campaigns performed within one month, revealed pervasive spread of B.1.1.7 with an average prevalence of 82%. Labs can easily implement this test to rapidly scale B.1.1.7 surveillance efforts and it is particularly useful in countries with high prevalence of variants possessing only the ΔH69/ΔV70 deletion because current strategies using target failure assays incorrectly identify these as putative B.1.1.7 variants.

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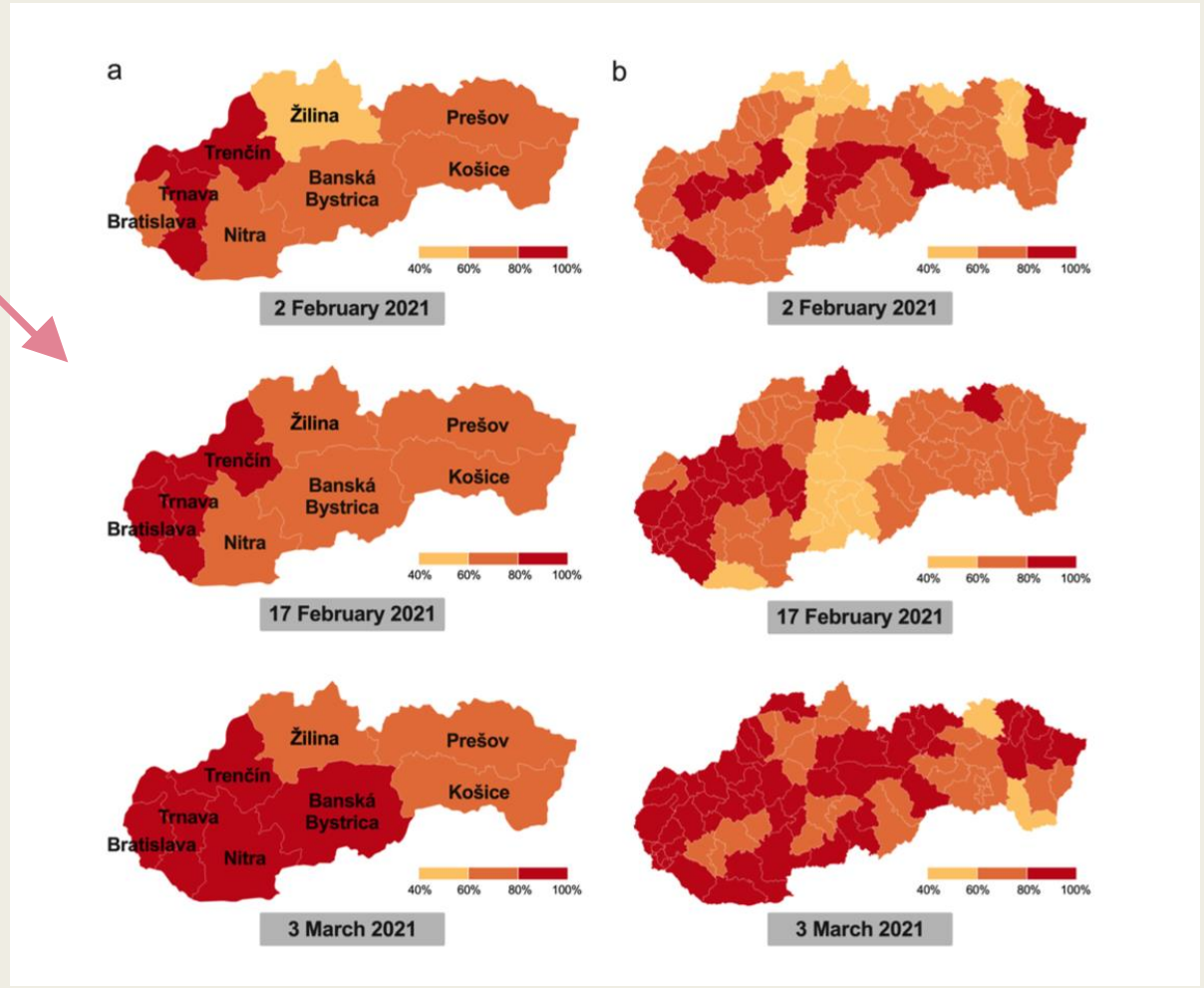
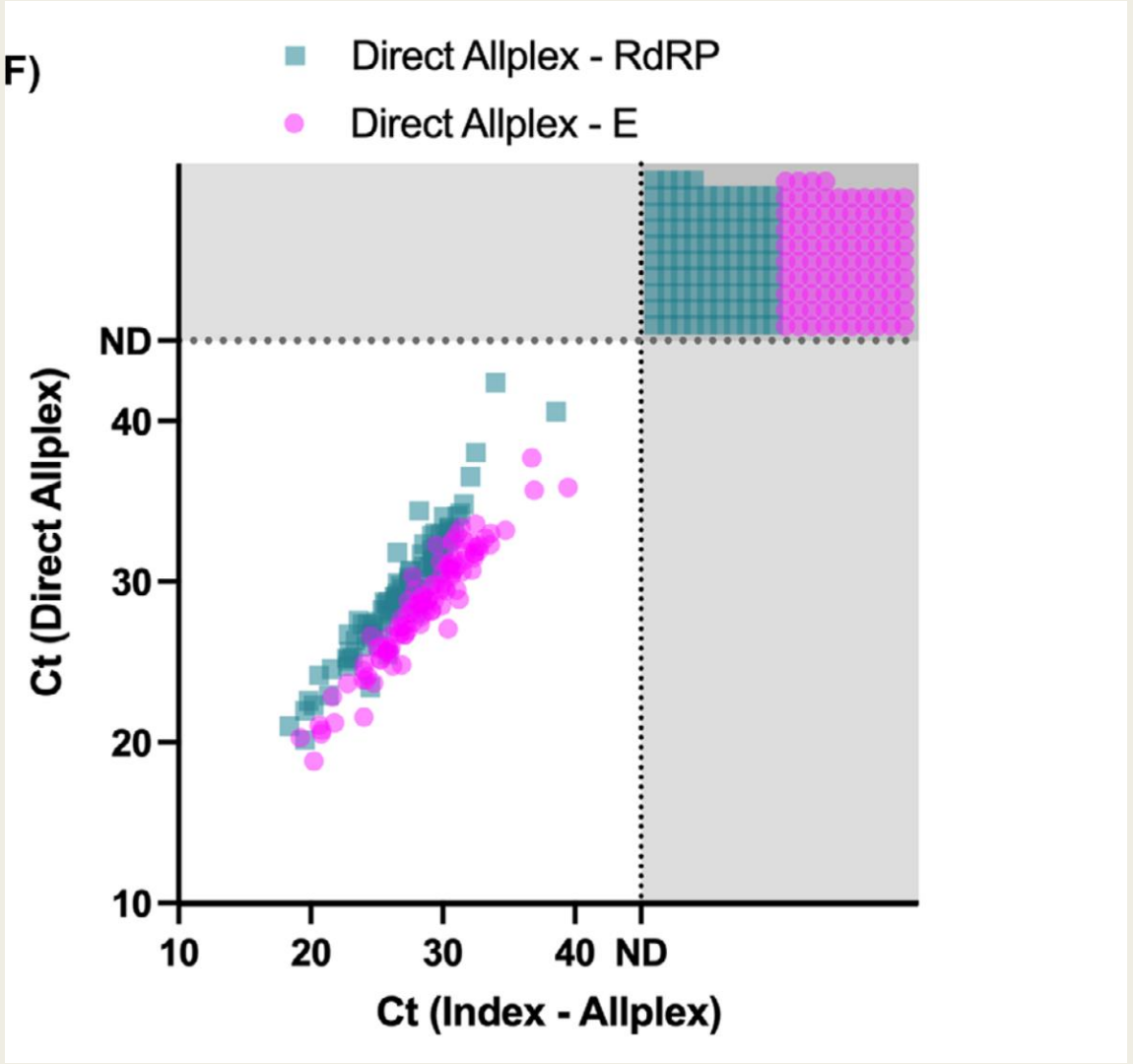


Tracking the prevalence of lineage B.1.1.7 in Slovakia. during the three screening rounds held on February 2nd, 2021, February 17th, 2021, and March 3rd, 2021.

rTEST COVID-19 qPCR Rapid Kit

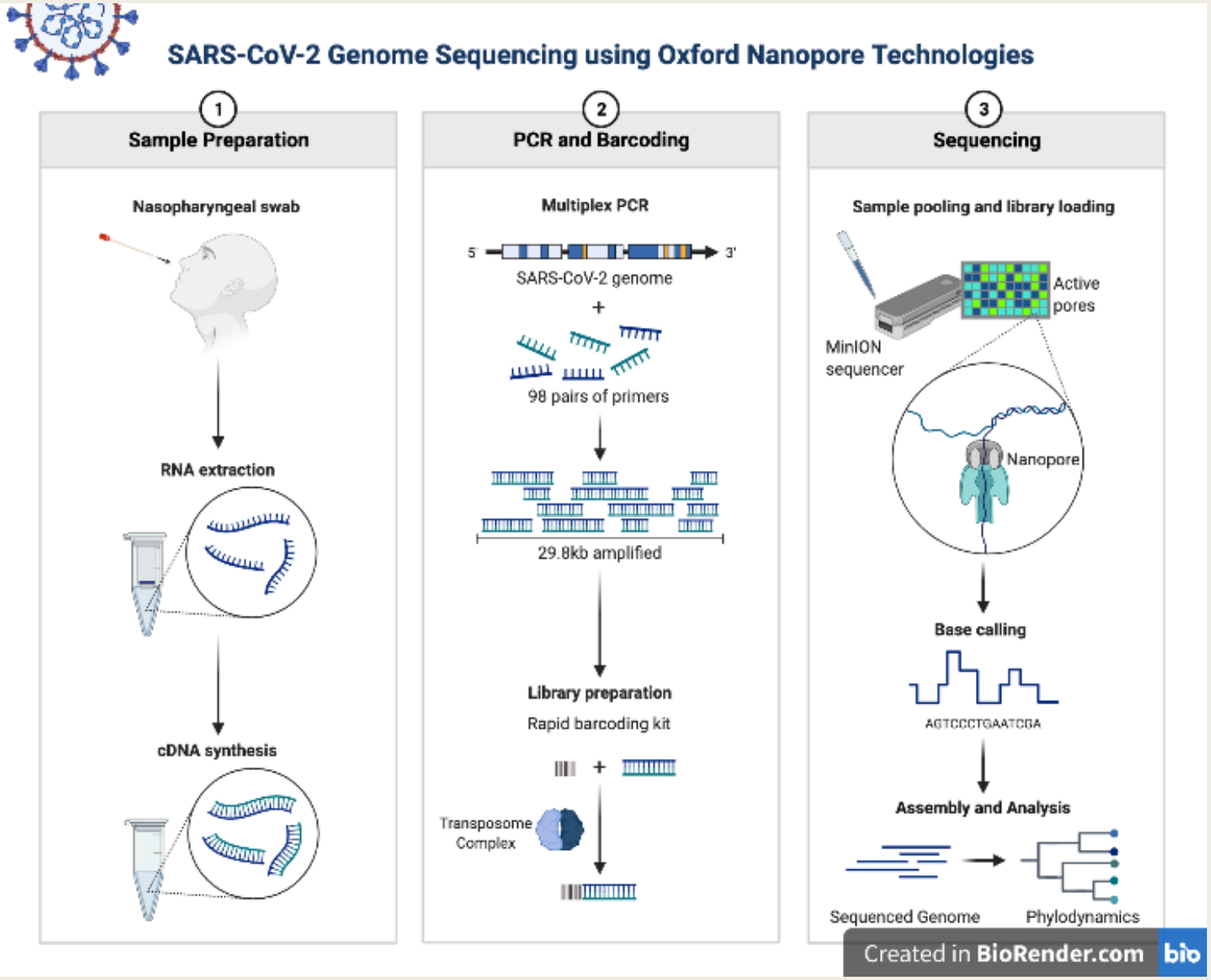
without RNA extraction step

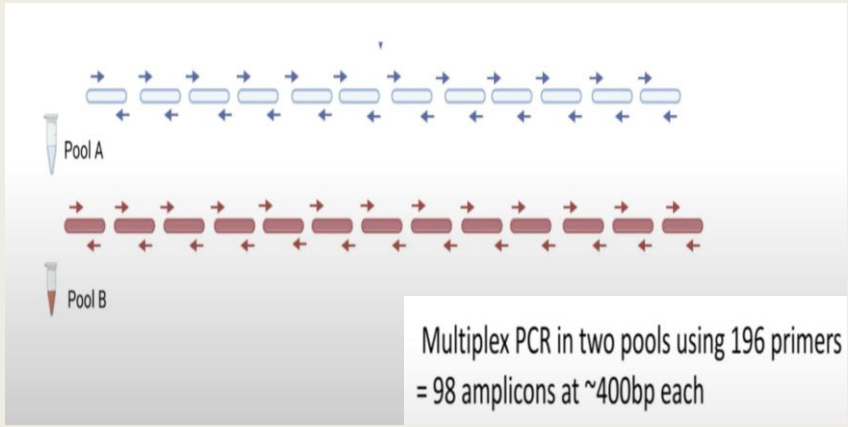
<u>Reaction time: 57 minutes</u>
100% diagnostic senzitivity
100% specificity



Clinical performance of the rTEST COVID-19 qPCR Rapid kit.

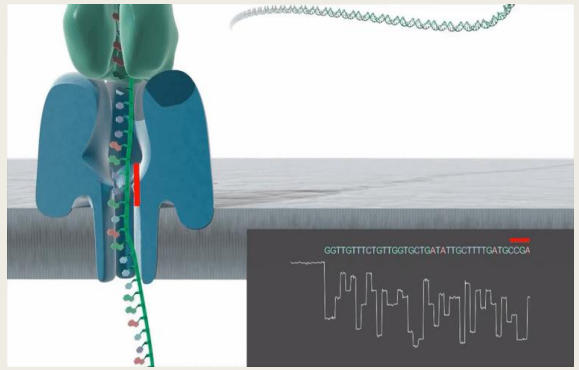
Sequencing work flow

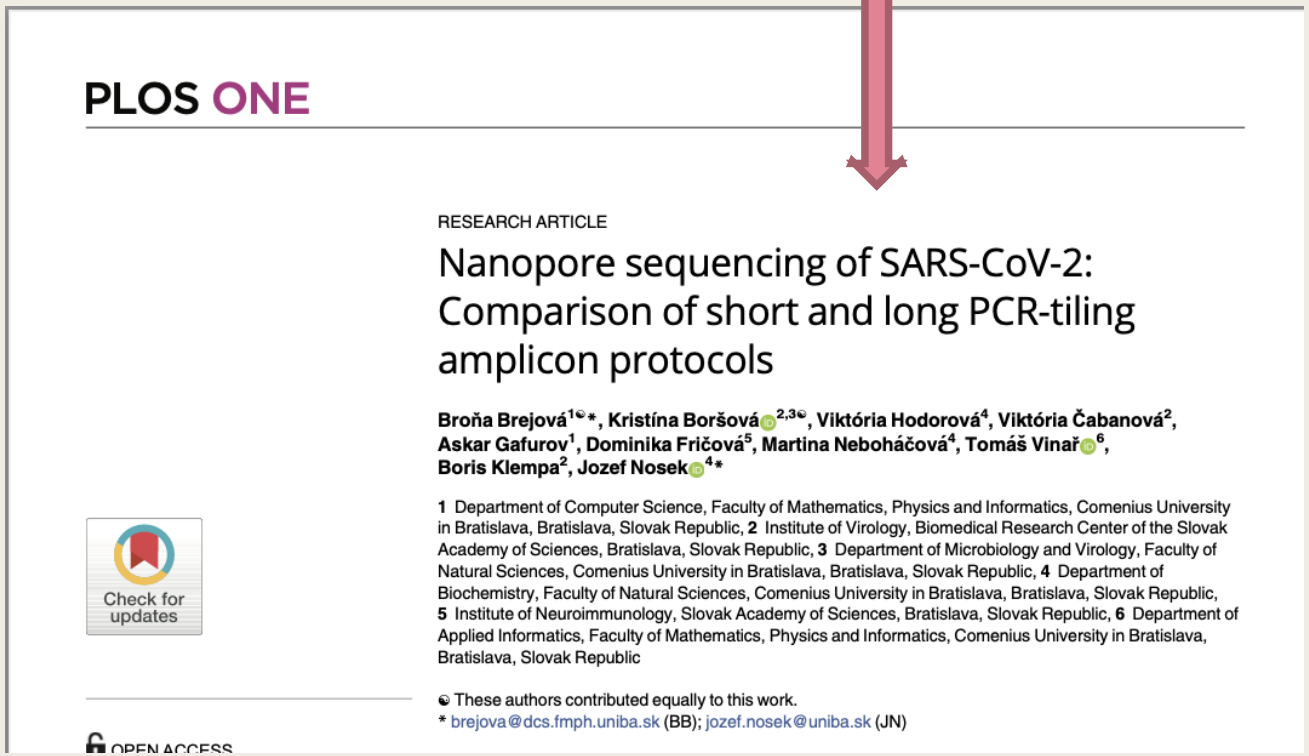




Sequencing of SARS-CoV-2 samples

- Beginning of sequencing : **July 2020**
- Use of **nanopore sequencing** methods (Oxford nanopore technology)
- **PCR tiling protocol- using 400bp, 2kb and 2,5kb primers sets**





PLOS ONE

RESEARCH ARTICLE

Nanopore sequencing of SARS-CoV-2: Comparison of short and long PCR-tiling amplicon protocols

Broňa Brejová[1☯]*, Kristína Boršová[2,3☯], Viktória Hodorová[4], Viktória Čabanová[2], Askar Gafurov[1], Dominika Fričová[5], Martina Neboháčová[4], Tomáš Vinař[6], Boris Klempa[2], Jozef Nosek[4]*

1 Department of Computer Science, Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava, Bratislava, Slovak Republic, **2** Institute of Virology, Biomedical Research Center of the Slovak Academy of Sciences, Bratislava, Slovak Republic, **3** Department of Microbiology and Virology, Faculty of Natural Sciences, Comenius University in Bratislava, Bratislava, Slovak Republic, **4** Department of Biochemistry, Faculty of Natural Sciences, Comenius University in Bratislava, Bratislava, Slovak Republic, **5** Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovak Republic, **6** Department of Applied Informatics, Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava, Bratislava, Slovak Republic

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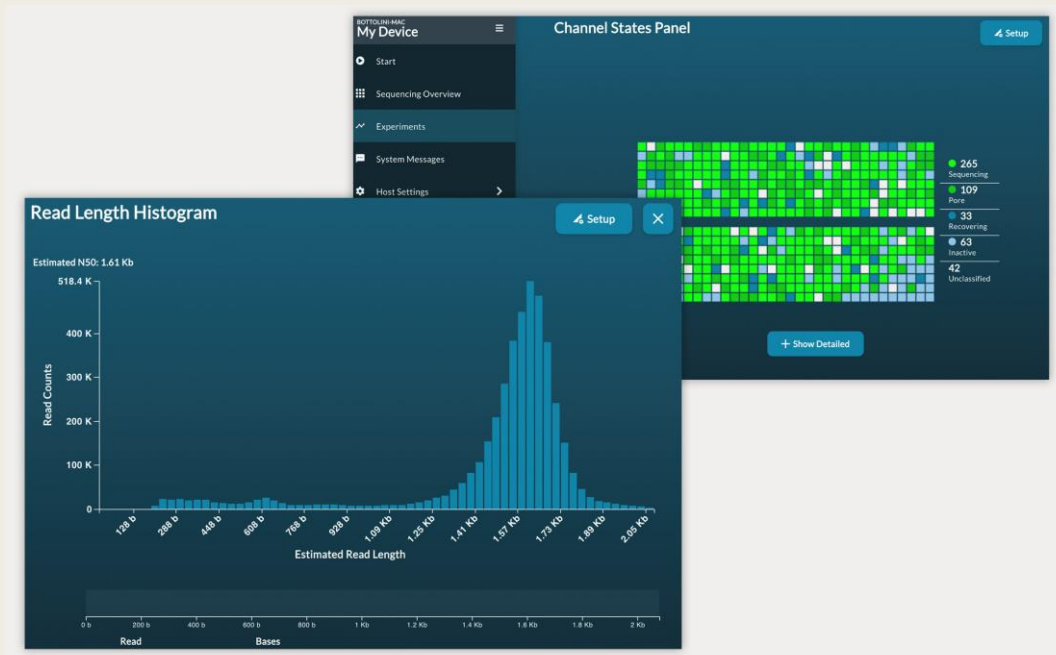
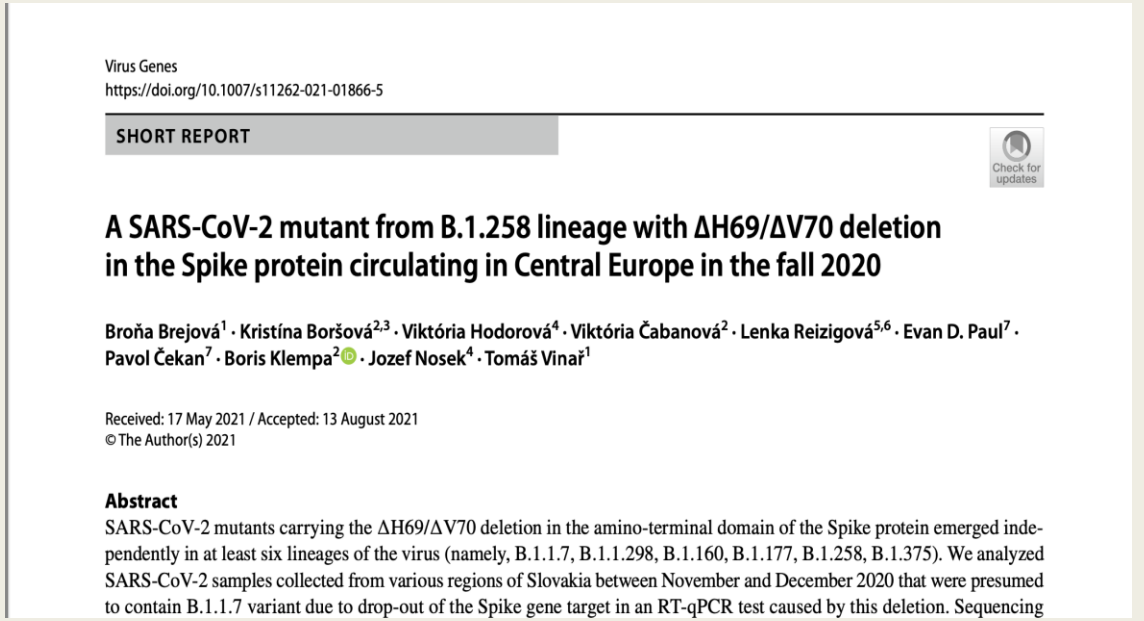


Sequencing between July 2020 – February 2022

- 6 100 samples
- 4 465 samples in the GISAID database
- 73,2 % sequencing success
- 27 variants



Virus Genes
https://doi.org/10.1007/s11262-021-01866-5

SHORT REPORT

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A SARS-CoV-2 mutant from B.1.258 lineage with ΔH69/ΔV70 deletion in the Spike protein circulating in Central Europe in the fall 2020

Broňa Brejová[1] · Kristína Boršová[2,3] · Viktória Hodorová[4] · Viktória Čabanová[2] · Lenka Reizigová[5,6] · Evan D. Paul[7] · Pavol Čekan[7] · Boris Klempa[2] · Jozef Nosek[4] · Tomáš Vinař[1]

Received: 17 May 2021 / Accepted: 13 August 2021

Abstract
SARS-CoV-2 mutants carrying the ΔH69/ΔV70 deletion in the amino-terminal domain of the Spike protein emerged independently in at least six lineages of the virus (namely, B.1.1.7, B.1.1.298, B.1.160, B.1.177, B.1.258, B.1.375). We analyzed SARS-CoV-2 samples collected from various regions of Slovakia between November and December 2020 that were presumed to contain B.1.1.7 variant due to drop-out of the Spike gene target in an RT-qPCR test caused by this deletion. Sequencing

First identifications:

Fall 2020: **B.1.258** variant (spread in central Europe)

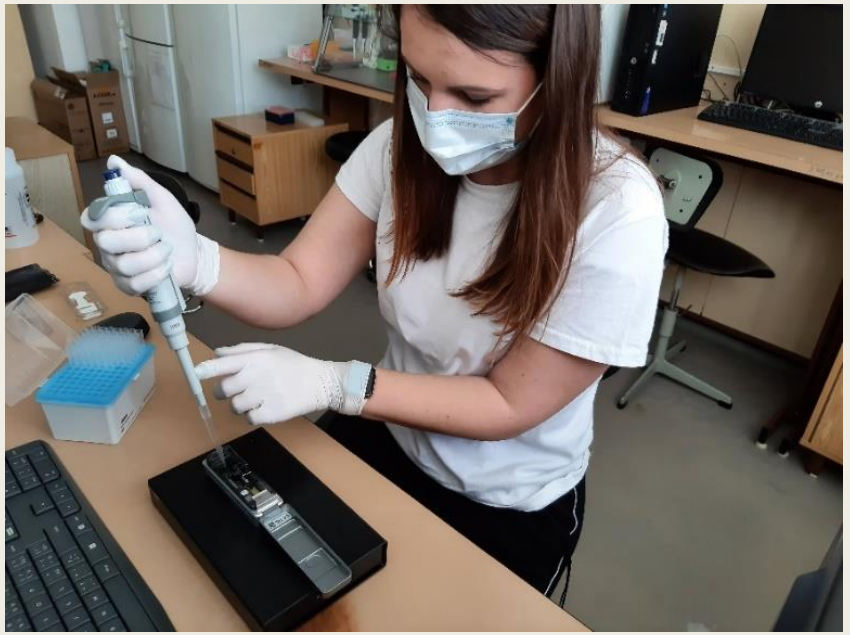
January 2021: **Alpha** variant (B.1.1.7)

March 2021: **Beta** variant (B.1.351)

June 2021: B.1.621 (Kolumbia, VOI)

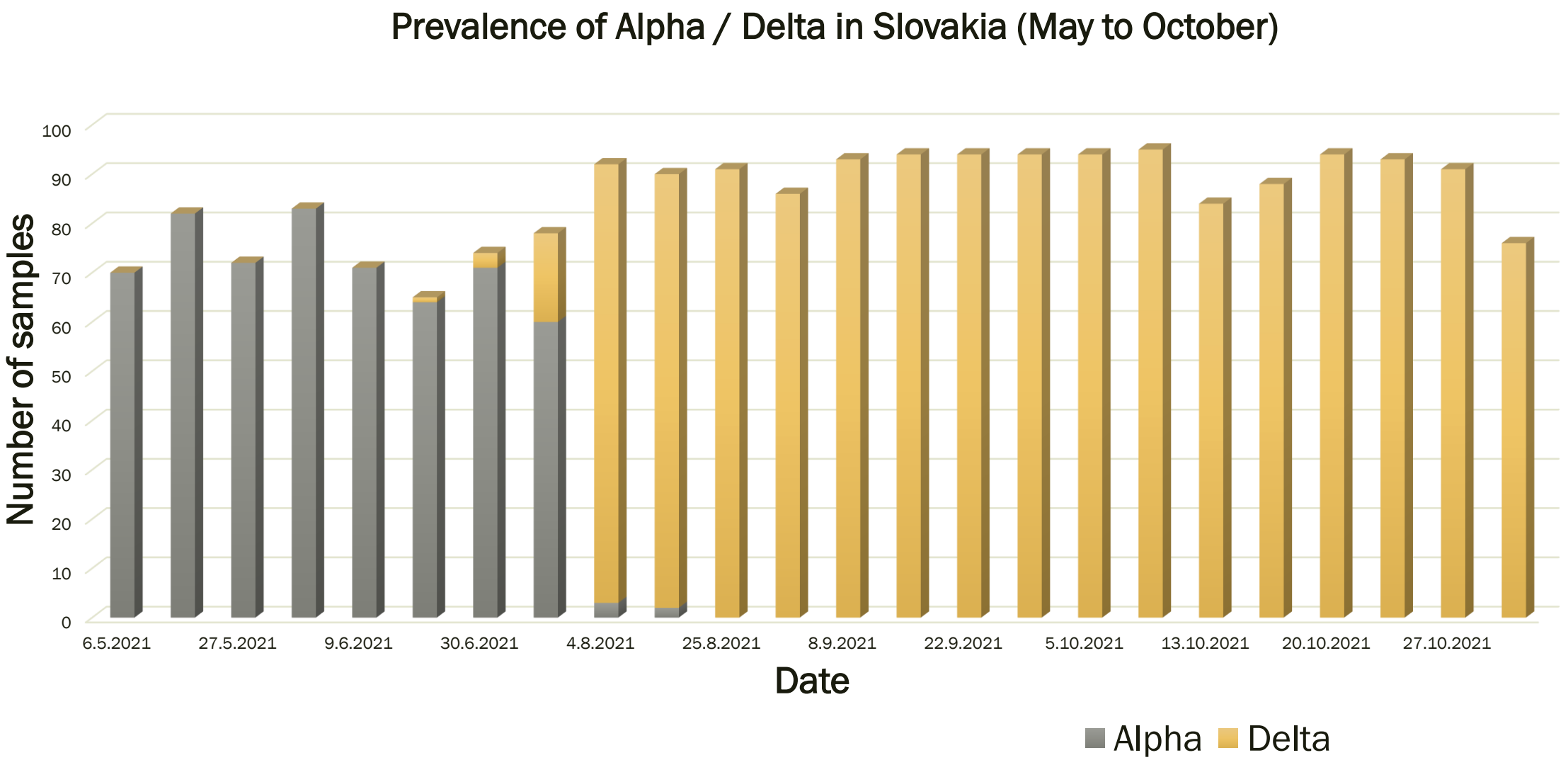
June 2021: **Delta** variant (B.1.617.2)

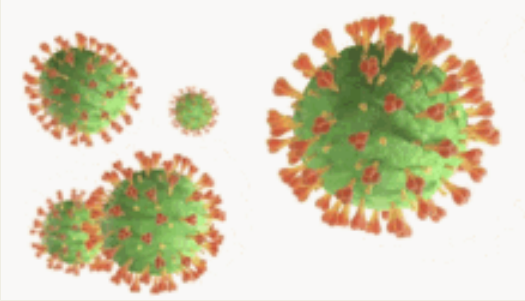
November 2021: **Omicron** variant (BA.1)



Quick switching of variants

Based on BMC sequencing





Sequencing requires close collaboration between several scientific disciplines...

- Biomedical research center SAS (sequencing)
- Department of Computer Science, Faculty of Mathematics, Physics and Informatics, Comenius University (bioinformatic analysis)
- Department of Biochemistry, Faculty of Natural Sciences, Comenius University (creation of protocols and troubleshooting)
- Public health authorities (samples source)

BIOMEDICAL RESEARCH CENTER SLOVAK ACADEMY OF SCIENCES

FACULTY OF MATHEMATICS, PHYSICS AND INFORMATICS
Comenius University
Bratislava

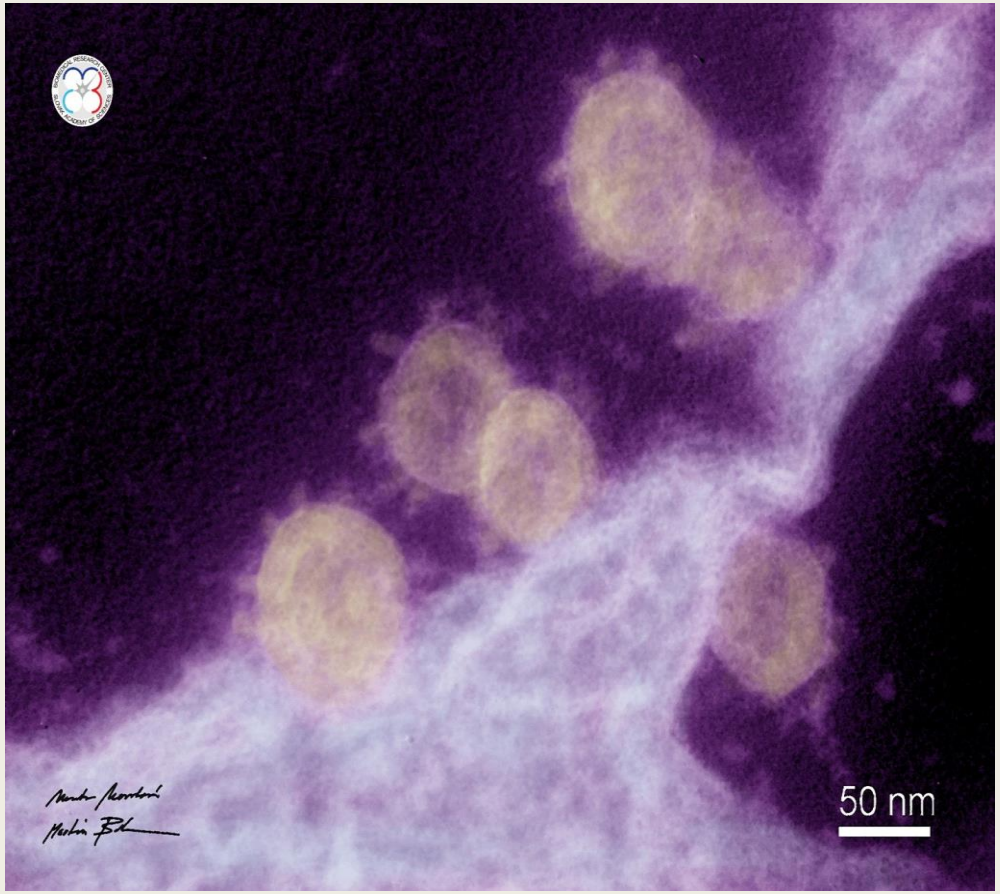
UNIVERSITAS COMENIANA BRATISLAVENSIS FACULTAS RERUM NATURALIUM MCMXIX

PUBLIC HEALTH AUTHORITY OF THE SLOVAK REPUBLIC

Summary and conclusions

Impact of the COVID 19 pandemic:

- disruption of scientific work and my PhD. study
- **opportunity to:**
  - ❖ help the society in the fight against the pandemic
  - ❖ obtain experience in applied research
  - ❖ promote importance of science
  - ❖ demonstrate that a multidisciplinary approach involving close **collaboration** between academia, government public health authorities and private biotechnology companies provides **great benefits to the society**



THANK YOU