V4 Young Researcher Award

Mgr. Kristína Boršová, PhD.
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

- **Laboratory confirmation**
  - RT-qPCR (gold standard)
  - Antigen tests

RT-qPCR - Detection the presence of one or more viral genes in a biological specimen.
  - RdRp gene (RNA dependent 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)
**Other viruses:**
- HCoV-229E
- HCoV-OC43
- HCoV-NL63
- 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

Primary screening | Reference method | rTEST COVID-19 RT-qPCR kit

CT value

RdRp | E gene | RdRP | E gene | RdRP | RNaseP

CT cutoff value
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 genes 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.
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

Reaction time: 57 minutes
100% diagnostic sensitivity
100% specificity

Clinical performance of the rTEST COVID-19 qPCR Rapid kit.
### SARS-CoV-2 Genome Sequencing using Oxford Nanopore Technologies

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<th>Step</th>
<th>Process</th>
<th>Image</th>
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<td>1</td>
<td>Sample Preparation</td>
<td><img src="image1.png" alt="Sample Preparation" /></td>
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<td>Nasopharyngeal swab</td>
<td><img src="image2.png" alt="Nasopharyngeal swab" /></td>
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<td>RNA extraction</td>
<td><img src="image3.png" alt="RNA extraction" /></td>
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<td>cDNA synthesis</td>
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<td>PCR and Barcoding</td>
<td><img src="image5.png" alt="PCR and Barcoding" /></td>
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<td>Multiplex PCR</td>
<td><img src="image6.png" alt="Multiplex PCR" /></td>
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<tr>
<td></td>
<td>98 pairs of primers</td>
<td><img src="image7.png" alt="98 pairs of primers" /></td>
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<td>29.8kb amplified</td>
<td><img src="image8.png" alt="29.8kb amplified" /></td>
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<td>Library preparation</td>
<td><img src="image9.png" alt="Library preparation" /></td>
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<td>Rapid barcoding kit</td>
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<td>3</td>
<td>Sequencing</td>
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<td>Sample pooling and library loading</td>
<td><img src="image12.png" alt="Sample pooling and library loading" /></td>
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<td>Multiplex PCR in two pools using 196 primers</td>
<td><img src="image13.png" alt="Multiplex PCR in two pools" /></td>
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<td>98 amplicons at ~400bp each</td>
<td><img src="image14.png" alt="98 amplicons" /></td>
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<td>Base calling</td>
<td><img src="image15.png" alt="Base calling" /></td>
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<td>Assembly and Analysis</td>
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<td>Sequenced Genome</td>
<td><img src="image17.png" alt="Sequenced Genome" /></td>
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<td>Phylogenetics</td>
<td><img src="image18.png" alt="Phylogenetics" /></td>
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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
• 6 100 samples
• 4 465 samples in the GISAID database
• 73,2 % sequencing success
• 27 variants

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

Prevalence of Alpha / Delta in Slovakia (May to October)

Number of samples

Date


Alpha Delta

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)
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