PRESS WORKSHOP
Sensitivity of Antigen Tests to the Omicron Variant

Exemplary Analysis by the Paul-Ehrlich-Institut (PEI), Robert Koch-Institut (RKI), Bundeswehr Institute of Microbiology (InstMikroBioBw)

Manufacturer Inquiry by the BfArM
AGENDA

Sensitivity of Antigen Tests
Exemplary Analysis by the Paul-Ehrlich-Institute, Robert Koch-Institute, Bundeswehr Institute of Microbiology

- Structure of the exemplary experimental test
  Prof. Klaus Cichutek

- Results
  PD Dr. Micha Nübling

- Manufacturer information about test design
  PD Dr. Micha Nübling

- Discussion
  Dr. Scheiblauer et al.
Omicron variant from Botswana and South Africa (Dec. 2021)

- More than 30 mutations in the spike protein with amino acid exchange
  - Immune evasion, increased transmission rate, better reproductive capacity
- 4 amino acid changes in the nucleocapsid protein
  - 2 of which have already been seen in earlier circulating variants

Target antigen of antigen assays

- 98.8% nucleocapsid protein
- 1.2% spike protein or spike protein + nucleocapsid protein
Omicron detection put in question by current antigen testing?

Diagnostic tests and Omicron:

- **US FDA** (Dec 2021): possible impaired detection of Omicron
  - no data published on rapid antigen testing
  - no known regulatory consequences (March 2022)

- **Bekliz,…, Eckerle** (2022):
  "Sensitivity of some Ag RDT reduced with Omicron"
  - Cell culture: Comparison of different SARS-CoV-2 variants (PFU)
  - Clinical samples (Delta/Omicron): 4/7 tests affected

- **Ostermann, …, Keppler** (2022): "Impaired Detection of Omicron"
  - Cell culture: no impairment to detection
  - Clinical samples (Delta/Omicron): 10 to 100-fold impaired detection
Analyses for the detection of the Omicron variant by antigen testing - test procedure

- **Investigation** of an exemplary sample of 20 rapid antigen tests, representative of the sensitivity range of positively evaluated tests
  - Swab
    - Pools of Patient Swabs (RKI)
      - Delta versus Omicron variant
  - Cell culture supernatant
    - Beta-propiolactone inactivated (InstMikroBioBw)
      - Wuhan versus Omicron variant

- **Transfer** of the results to further tests ("bridging" on the basis of similar test designs)
Clinical samples positive for Delta and Omicron are detected comparably well

Clinical samples (swab pools; RKI), visual reading of antigen test results

- 4x delta concentrations corresponding to Ct 24.4 to 32.1
- 4x Omicron concentrations according to Ct 23.2 to 32.6

<table>
<thead>
<tr>
<th>Pool #</th>
<th>ct E-Gen</th>
<th>Delta</th>
<th>Omikron</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta 1</td>
<td>24,39</td>
<td>+ + + + + + + + + + + + + (+) (+)</td>
<td>+</td>
</tr>
<tr>
<td>Delta 2</td>
<td>25,59</td>
<td>+ + + + + + + + (+) (+) + + + (+) (+)</td>
<td>- +</td>
</tr>
<tr>
<td>Delta 3</td>
<td>29,19</td>
<td>(+) (+) + + - - + - - (+) - - - (+) (+)</td>
<td>- - -</td>
</tr>
<tr>
<td>Delta 4</td>
<td>32,07</td>
<td>- - - (+) - - - - - - - - (+)</td>
<td>- - -</td>
</tr>
</tbody>
</table>

Omicron

| Omicr A | 23,18 | + + + + + + + + + (+) (+) + + + + | (+) (+) + |
| Omicr B | 25,77 | + + + + + + + + - (+) + (+) + - (+) | + + |
| Omicr C | 28,68 | + + + + + + (+) + (+) - - - - (+) - (+) - (+) | - |
| Omicr D | 32,63 | (+) (+) + + + (+) - - (+) - - - - (+) | - - - |
Cell culture samples positive for Wuhan and Omicron are detected comparably well

Cell culture samples (RKI, PEI, InstMikroBioBw), reading band intensity (scanner)

- 6 Wuhan concentrations corresponding to $10^8$ to $10^4$ virus particles
- 6 Omicron concentrations corresponding to $10^8$ to $10^4$ virus particles

### Rapid antigen test

<table>
<thead>
<tr>
<th>Nr</th>
<th>Verdi</th>
<th>LoD RNA</th>
<th>Wildtyp-Virus (Wuhan)</th>
<th>Omikron</th>
<th>LoD RNA</th>
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</thead>
<tbody>
<tr>
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<tr>
<td>1</td>
<td>8</td>
<td>3,5E+07</td>
<td>336 359 385 387 185 308 179 212 287 158 70 157 139 216 130 194 82</td>
<td>1,9E+07 333 340 381 352 194 350 249 270 293 296 135 193 159 190 232 154 107</td>
<td>5,1E+04 7,3E+04 4,2E+04 6,0E+04 2,9E+05 2,1E+05 2,4E+05 6,2E+05 2,9E+05 4,6E+05 3,8E+05 2,9E+05 9,5E+05 7,3E+05 4,7E+05 4,7E+05 9,5E+05 2,9E+05 8,2E+05 8,8E+05</td>
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<td>2</td>
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<td>4,7E+06 211 269 333 277 121 152 102 87 77 91 60 67 37 60 60 64 16</td>
<td>1,4E+05 8,5E+04 7,3E+04 3,8E+05 2,7E+05 7,0E+05 1,3E+06 9,6E+05 9,3E+05 4,4E+05 4,4E+05 1,4E+06 1,1E+06 1,1E+06 1,3E+06 1,8E+06 4,4E+05 7,3E+05 7,8E+05</td>
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<tr>
<td>3</td>
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<td>40 162 143 189 41 48 13 7 12 16 11 6 5 5 7 4 14</td>
<td>1,4E+05 8,5E+04 7,3E+04 4,2E+04 6,0E+04 2,9E+05 2,1E+05 2,4E+05 6,2E+05 2,9E+05 4,6E+05 3,8E+05 2,9E+05 9,5E+05 7,3E+05 4,7E+05 4,7E+05 9,5E+05 2,9E+05 8,2E+05 8,8E+05</td>
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<tr>
<td>4</td>
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<td>8 17 25 52 5 9 2 0 0 0 4 4 0 2 2 0 0 4</td>
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<td>1,4E+05 8,5E+04 7,3E+04 3,8E+05 2,7E+05 7,0E+05 1,3E+06 9,6E+05 9,3E+05 4,4E+05 4,4E+05 1,4E+06 1,1E+06 1,1E+06 1,3E+06 1,8E+06 4,4E+05 7,3E+05 7,8E+05</td>
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<td>1,4E+05</td>
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<td>5,1E+04 7,3E+04 4,2E+04 6,0E+04 2,9E+05 2,1E+05 2,4E+05 6,2E+05 2,9E+05 4,6E+05 3,8E+05 2,9E+05 9,5E+05 7,3E+05 4,7E+05 4,7E+05 9,5E+05 2,9E+05 8,2E+05 8,8E+05</td>
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<tr>
<td>6</td>
<td>8,192</td>
<td>3,4E+04</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
<td>1,9E+07 333 340 381 352 194 350 249 270 293 296 135 193 159 150 232 154 107</td>
<td>5,1E+04 7,3E+04 4,2E+04 6,0E+04 2,9E+05 2,1E+05 2,4E+05 6,2E+05 2,9E+05 4,6E+05 3,8E+05 2,9E+05 9,5E+05 7,3E+05 4,7E+05 4,7E+05 9,5E+05 2,9E+05 8,2E+05 8,8E+05</td>
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</tbody>
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Paul-Ehrlich-Institut

Prof. Dr. Klaus Cichutek, PD Dr. Micha Nübling, Dr. Heinrich Scheiblauer
Ranking of individual antigen test sensitivity to Omicron corresponds to the ranking determined against Wuhan.
No evidence of decreased sensitivity of antigen tests to Omicron

- Investigations by the Paul-Ehrlich-Institut do not indicate reduced sensitivity of rapid antigen tests to Omicron
  - Sensitivity to Omicron analogous to that of previous SARS-CoV-2 variants (Wuhan, Delta)
  - confirmed by other publications

- Results of the Paul-Ehrlich-Institut study can be applied to further tests of similar designs
Further investigations confirm Omicron detection by antigen tests

- Nielsen et al (Statens Serum Institut Copenhagen, DK)
  - 9 different Ag RDTs, different variants (cell culture)
- Goderski et al. (RIVM, NL)
  - 7 different Ag RDTs, Omicron versus Wuhan (cell culture)
- UK Health Security Agency
  - 5 different Ag RDTs, Omicron versus Wuhan (cell culture)
- WHO Prequalification Workshop (02/2021)
  - no known reduced sensitivity in pre-qualified tests
  - 10 Ag RDTs similar for Delta and Omicron
- Molenkamp, R & Igloi, Z.
  - 3 Ag RDTs Omicron vs Delta
- Barbara L Goodall (2022)
  - Omicron comparable to evaluations with other SARS-CoV-2 strains
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- Manufacturer information on the test design (BfArM inquiry)
  PD Dr Nübling

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  Dr Scheiblauer et al.
Mutations in the nucleocapsid protein of SARS-CoV-2 variants
- Design of most antigen tests aims at N-protein recognition -

**Wuhan** – December 2019

**Gamma** – December 2020

**Delta** – December 2020

**Omicron** – November 2021

**Mutations** in the nucleocapsid protein
Various SARS-CoV-2 variants
The tests evaluated on an exemplary basis at the Paul-Ehrlich-Institut use conserved regions of the nucleocapsid protein.

Omicron (BA1, BA2): *Mutations* in the nucleocapsid protein

![Diagram showing conserved regions of the nucleocapsid protein]
Information on the test design allows for an assessment of Omicron detection

Inquiry of the BfArM on the design of all rapid antigen tests

- Antibodies (usually mAb; capture, detection)
  - Binding region of antibodies
- 428 evaluable answers (23/03/2022)
  - 385 tests (90%) with Ak binding region without Omicron mutations
  - 43 tests (10%) may be affected by Omicron mutation
    - Validation of Omicron detection by manufacturers
Information on the test design allows for an assessment of Omicron detection.

**Transfer** to further tests ("bridging" based on similar designs)

Omicron (BA1, BA2): **Mutations** in the nucleocapsid protein

![Diagram showing mutations in the nucleocapsid protein](image.png)

- N-arm: 310 tests
- NTD: 19
- Linker: 56 tests
- CTD: 22 tests
- C-tail: 10
- Total: 11
Investigations by the Paul-Ehrlich-Institut do not indicate reduced sensitivity of rapid antigen tests to Omicron.

BfArM inquiry provides information on test design (antibody target regions in N):
- Antibody binding region outside of mutated regions
  - on BfArM list; reimbursable
- Antibody binding region (potentially) overlapping with mutations
  - Manufacturer validation of Omicron detection
- Removal of tests without reliable information

BfArM list (recoverable antigen tests): information on test design now included:
- Bridging: 'yes' = target region of the antibodies used outside mutated N regions
The information on Omicron detection has been included on the BfArM list since 23.03.2022.

### Antigen-Tests auf SARS-CoV-2 zur professionellen Anwendung

Die Gegenstand des Anspruchs nach §1 Satz 1 Coronavirus-Testverordnung (TestV) sind („Schnelltests“)

<table>
<thead>
<tr>
<th>Test-ID</th>
<th>Handelsname</th>
<th>Evaluierung PEI</th>
<th>Omikron-Erkennung entsprechend der Bridging-Prüfung des PEI</th>
<th>Hersteller</th>
<th>Stadt</th>
<th>Land</th>
<th>Name</th>
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<tbody>
<tr>
<td>AT025/22</td>
<td>SMARTTEST SARS-COV-2 NASOPHARYNGEAL ANTIGEN RAPID TEST</td>
<td>Nein</td>
<td>Nein</td>
<td>A PLUS DIAGNOSTICS LAB.SAN.TIC.A.S.</td>
<td>Ümraniye/istanbul</td>
<td>TR</td>
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<tr>
<td>AT005/20</td>
<td>Panbio™ COVID-19 Ag Rapid Test Device (Nasopharyngeal)</td>
<td>Ja</td>
<td>Ja</td>
<td>Abbott Rapid Diagnostics Jena GmbH</td>
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<td>DE</td>
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<td>AT116/21</td>
<td>Panbio™ COVID-19 Ag Rapid Test Device (Nasal)</td>
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<td>Ja</td>
<td>Abbott Rapid Diagnostics Jena GmbH</td>
<td>Jena</td>
<td>DE</td>
<td></td>
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Future regulation of SARS-CoV-2 diagnostics - Outlook -

- **New IVD regulation** with stricter regulations enters into force on 26.05.2022

- **Certification** of new SARS-CoV-2 diagnostics only after
  - appraisal by Notified Body
  - Laboratory testing by an EU reference laboratory

- **Transitional provisions** permits previously (self) certified SARS-CoV-2 diagnostics until May 2025
Im Mittelpunkt steht die Gesundheit
Our Focus is on Health