# Evaluating diagnostic performance of SARS-CoV-2 AG-RDTs based on extensive data from field use

## *a data science approach*

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ECDC/WHO Euro joint lab network meeting

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# Goals

1. Determine sensitivity and specificity for most commonly used tests
   - 38 tests with at least 300 PCR- and 100 PCR+

2. Are AG-RDTs on approved lists better?
   - WHO Emergency Used Listing (2020, 22)
   - EU Common List of antigen tests
   - Paul Ehrlich Inst. evaluation (Germany)
   - UK Health Security Agency (DHSC/PHE evaluation)

3. Determine sensitivity and specificity for subgroups:
   - Most commonly used tests (38)
   - Sample type (saliva, nasal, nasoph.)
   - Age groups
   - Symptoms
   - According to SARS-CoV-2 incidence in region
   - Vaccinated/unvaccinated

# Outline

Motivation

- Verification of the clinical performance of AG-RDT used for population screening in the Czech republic

Methodology and data sources

- Records of state-wide field testing for COVID-19 in the Czech Republic
- Data enrichment

Results

Conclusions and recommendations

- Test sensitivity was lower for children and adolescents, vaccinated individuals, saliva tests, tests conducted for preventive reasons and in periods of low SARS-CoV-2 incidence
- Test approved on the WHO/ECDC/PHE lists performed better

# Motivation

# Motivation – divergence between declared diagnostic performance

Initial approach for selecting AG-RDTs for public use relied on sensitivities and specificities declared by manufacturers.

| | | | |
|---|---|---|---|
| [redacted] | COVID-19 Antigen Test Kit (Colloidal Gold) Clinical Performance Study Report | FM No: | CP-TED-15-05 |
| | | Rev No: | A/0 |

12.1 **Clinical performance**

| Method | | PCR results | | Total |
|---|---|---|---|---|
| | | Positive | Negative | |
| COVID-19 antigen Test kit | Positive | 167 | 1 | 168 |
| | Negative | 0 | 229 | 229 |
| Total | | 167 | 230 | 397 |
| Sensitivity | | >99.99% | 95% confidence interval | 97.75%~100% |
| Specificity | | 99.57% | 95% confidence interval | 97.58%~99.92% |
| Accuracy | | 99.75% | 95% confidence interval | 98.59%~99.96% |

# … and independent studies

- Independent evaluation of a version of COVID-19 test from the same manufacturer has shown a very different result

Paul-Ehrlich-Institut

| Hersteller / Manufacturer | Testname / Test name | Zielantigen / target antigen | Sensitivität / Sensitivity Cq ≤25 | Cq 25-30 | Cq ≥30 | Gesamt-Sensitvität / total sensitivity |
|---|---|---|---|---|---|---|
| [illegible] | [illegible] | N+S | 65,0% | 5,0% | 0,0% | 28,0% |
| [illegible] | [illegible] | N | 0,0% | 0,0% | 0,0% | 0,0% |

- Average AG-RDT sensitivity according to a metastudy of 112,000 samples was **71.2%** (Brümmer et al, 2021)

Source: Results of the Comparative Evaluation of the Sensitivity of SARS-CoV-2 Antigen Rapid Tests, Paul Ehrlich Institut, 30 May 2022

# Excerpt from manufacturer clinical study of the CE-certified AG-RDT

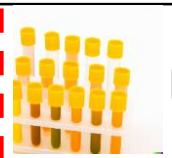
| Testing Date | 2021.03.02~2021.03.11 | | | |
|---|---|---|---|---|
| Testing Method | PCR results | | Total | Remarks |
| COVID-19 antigen Test kit | Positive | Negative | | |
| Positive | 0 | 1 | 1 | Annex 4 |
| Negative | 0 | 196 | 196 | |
| Total | 0 | 197 | 197 | |

Negative cases evaluated in China on PCR-negative cases

| Testing Date | 2021.03.04~2021.03.06 | | | |
|---|---|---|---|---|
| Testing Method | PCR results | | Total | Remarks |
| COVID-19 antigen Test kit | Positive | Negative | | |
| Positive | 80 | 0 | 80 | Annex 1 |
| Negative | 0 | 0 | 0 | |
| Total | 80 | 0 | 80 | |

Positive cases evaluated in Equador on PCR-positive cases

# AG-RDT evaluations with existing methodologies

| Type of AG-RDT evaluation | | |
|---|---|---|
|  Clinical study by AG-RDT manufacturer |  Independent in-vitro study |  Independent prospective clinical field study |
| **Example AG-RDT approval lists using the evaluation type** | | |
| CE mark | EU Common List Category B | EU Common List Category A |
| **Limitations** | | |
| • Not independent<br>• Not periodic<br>• Mutually incomparable<br>• Sometimes problematic access to COVID-19 positive cases (e.g., Chinese manufacturers) | • Not periodic<br>• Issue with batches<br>• Results may not match with clinical evaluations (e.g., mutation in frozen sample pools no longer in circulation)<br>• Limited sample size | • Incomparable: individual AG-RDTs evaluated on different populations across involved countries<br>• Not periodic: does not capture differences among batches or versions of tests distributed in different countries<br>• Subpopulations not evaluated: important target subgroups such as children or preventive testing may behave differently |

BETTER

# Our methodology and data sources

# Our methodology: Pairing of AG-RDTs and PCRs registered as part of regular state-mandated AG-RDT and PCR testing

Reasons for state-mandated testing

Data from state-wide official AG-RDT testing

- Preventive (screening)
- Diagnostic (susp. infection)
- Epidemiologic (contact tracing)

Data from state-wide official RT-PCR testing

Hundreds of testing sites log results into one central database


Photo Credit: ČRo, Denik.cz, DALL-E

Test results

**INFORMATION SYSTEM FOR INFECTIOUS DISEASES (ISIN)**

Patient data

RT-PCR, AG-RDT, results and patient information such as VACCINATION status are connected

# Glimpse of data

| Age | | | AG-RDT | | Days PCR after AG-RDT | Symptoms | | | | | | | | | AG-RDT vs PCR match | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Datum výsledku | Věková kategorie | Kód kraje | Kód testu | Název testu | Výrobce testu | Odstup PCR testu od AG testu | Typologie testu | Kašel | Bolesti svalů, kloubů, zimnice | Průjem/Zvracení | Teplota | Ztráta chuti, čichu | Jiné symptomy | Očkování | Prodělaný COVID | Hospitalizace | AG+ s násl. PCR+ | AG+ s násl. PCR- | AG- s násl. PCR+ | AG- s násl. PCR- |
| 1.12.2021 | 0-12 | CZ010 | | ######## | ######## | 2.den | 2 | | | | | | | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 1.12.2021 | 0-12 | CZ010 | | ######## | ######## | nasledujici den | 2 | | | | | | | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1.12.2021 | 0-12 | CZ010 | | ######## | ######## | stejny den | 1,5 | | | | | | | 0 | 0 | 0 | 1 | 1 | 0 | 1 |
| 1.12.2021 | 0-12 | CZ010 | | ######## | ######## | stejny den | 2 | | | | | | | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1.12.2021 | 0-12 | CZ010 | 1232 | Panbio Co | Abbott Ra | nasledujici den | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 1.12.2021 | 0-12 | CZ010 | 1375 | DIAQUICK | DIALAB Gr | 2.den | 1,5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1.12.2021 | 0-12 | CZ010 | 1437 | Wondfo 2 | Guangzho | nasledujici den | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1.12.2021 | 0-12 | CZ010 | 1468 | Flowflex S | ACON Lab | 3.den | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1.12.2021 | 0-12 | CZ010 | 1468 | Flowflex S | ACON Lab | nasledujici den | 1,5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1.12.2021 | 0-12 | CZ010 | 1468 | Flowflex S | ACON Lab | stejny den | 1,5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1.12.2021 | 0-12 | CZ010 | 1489 | COVID-19 | Safecare E | nasledujici den | 1,5 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 1.12.2021 | 0-12 | CZ010 | 1489 | COVID-19 | Safecare E | nasledujici den | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1.12.2021 | 0-12 | CZ010 | 1608 | ANTIGEN I | A. Menari | stejny den | 1,5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1.12.2021 | 0-12 | CZ010 | 1608 | ANTIGEN I | A. Menari | stejny den | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1.12.2021 | 0-12 | CZ010 | 1957 | COVID-19 | Zhuhai Lit | 2.den | 1,5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| 1.12.2021 | 0-12 | CZ010 | 2099 | VivaDiag F | VivaChek | 2.den | 1,5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1.12.2021 | 0-12 | CZ010 | 2099 | VivaDiag F | VivaChek | 2.den | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1.12.2021 | 0-12 | CZ010 | 2099 | VivaDiag F | VivaChek | nasledujici den | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 1.12.2021 | 0-12 | CZ010 | 2099 | VivaDiag F | VivaChek | stejny den | 1,5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 1.12.2021 | 0-12 | CZ010 | LepuAntig | Beijing Le | NULL | nasledujici den | 1,5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 1.12.2021 | 0-12 | CZ010 | LepuAntig | Beijing Le | NULL | nasledujici den | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| 1.12.2021 | 0-12 | CZ010 | LepuAntig | Beijing Le | NULL | stejny den | 1,5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1.12.2021 | 0-12 | CZ020 | | ######## | ######## | nasledujici den | 1,5 | | | | | | | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| 1.12.2021 | 0-12 | CZ020 | | ######## | ######## | nasledujici den | 1,5 | | | | | | | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| 1.12.2021 | 0-12 | CZ020 | 1223 | BIOSYNEX | BIOSYNEX | 2.den | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1.12.2021 | 0-12 | CZ020 | 1223 | BIOSYNEX | BIOSYNEX | 3.den | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| 1.12.2021 | 0-12 | CZ020 | 1232 | Panbio Co | Abbott Ra | 2.den | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 1.12.2021 | 0-12 | CZ020 | 1232 | Panbio Co | Abbott Ra | 2.den | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1.12.2021 | 0-12 | CZ020 | 1232 | Panbio Co | Abbott Ra | 2.den | 1,5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| 1.12.2021 | 0-12 | CZ020 | 1232 | Panbio Co | Abbott Ra | nasledujici den | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1.12.2021 | 0-12 | CZ020 | 1232 | Panbio Co | Abbott Ra | nasledujici den | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1.12.2021 | 0-12 | CZ020 | 1331 | SARS-CoV | Beijing Le | 2.den | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |

# Overview of data - Delta

| | Delta |
|---|---|
| Data collected from | 5 August 2021 |
| Data collected to | 6 December 2021 |
| AG-RDT types | ~450 |
| AG-RDT paired with positive PCRs. | 346,221 |
| True positives | 49,618 |
| False positives | 9,111 |
| False negatives | 18,961 |
| True negatives | 268,531 |

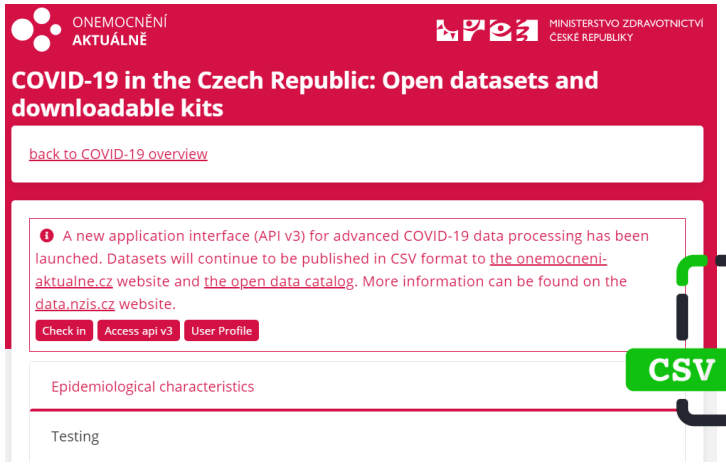
Preliminary

# Overview of data - Omicron vs Delta

We will also include not yet published results for Omicron.
For these comparisons, we represent Delta with the last recorded month of Delta prevalence according to discriminatory PCR and Omicron with the first month of prevalence:

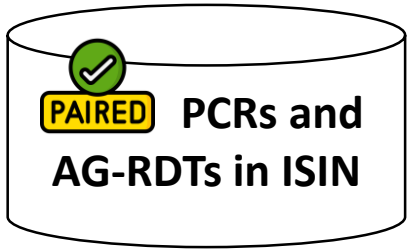
| | | Delta | | Omicron |
|---|---|---|---|---|
| Data collecting from | Left out | **1 Dec 21** | Left out | **20 Jan 22** |
| Data collecting to | | **25 Dec 22** | | **22 Feb 22** |
| AG-RDT paired with positive PCRs | Possible other factors affecting comparability with Omicron | 51,880 | There was a mix of variants according to discriminatory PCR tests | 116,530 |
| True positives | | 14,027 | | 42,383 |
| False positives | | 1,973 | | 5,330 |
| False negatives | | 4,000 | | 15,306 |
| True negatives | | 31,880 | | 53,511 |

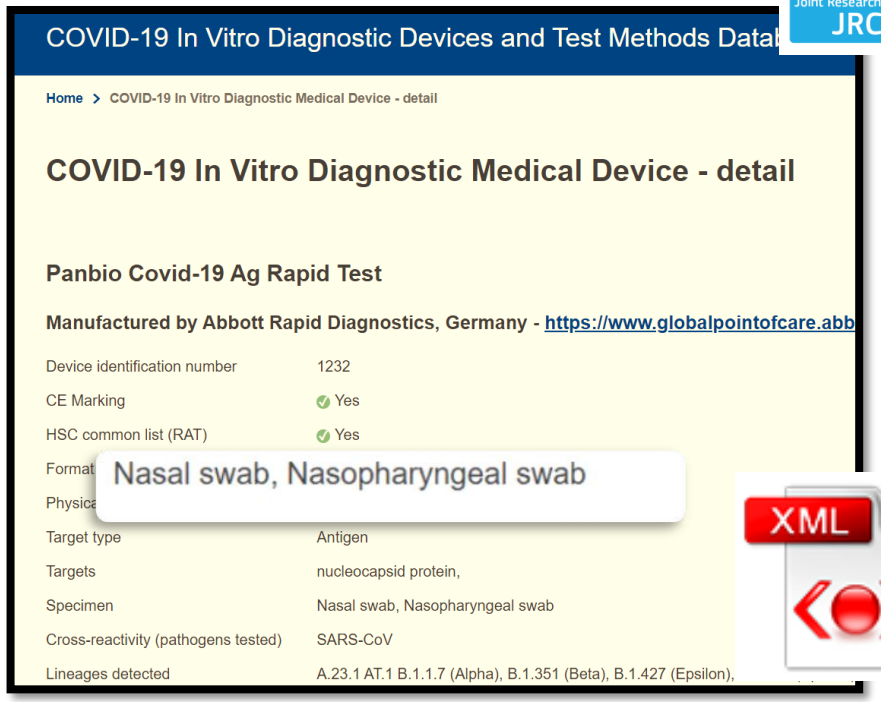
# Enriching data with external information




CSV

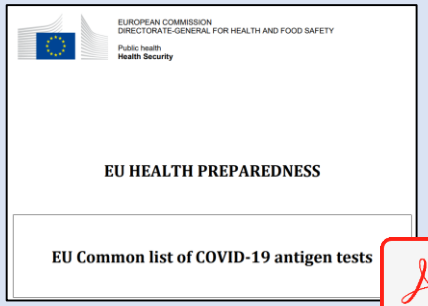
**Incidence of Sars-Cov-2**
in regions of Czechia




XML

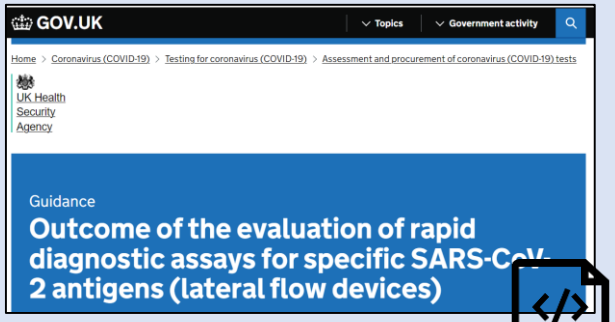
Information on sample type for AG-RDTs




PDF


PDF


PDF




HTML

Lists of approved AG-RDTs

# Data analysis

Three independent pipelines for different use cases

All allowing access for authenticated users via a web interface

MS EXCEL + Sharepoint

MS EXCEL + PowerBI


Python + Jupyter



Open-source software

# Overview of results

# Results – 38 individual AG-RDTs

- Included were AG-RDTs with at least 300 PCR- and  100 PCR+ samples
- Results were in-line with several previously published studies
- The best performing AG-RDT from the list of 38 tests was the same test as determined in metastudy of Brümmer et al., 2021*
- We observed that the same test had below-average sensitivity as reported to have inconsistent performance in Denzler et al., 2022
- Please refer to our article for details (*Role of population and test characteristics in antigen-based SARS-CoV-2 diagnosis, Czechia, August to November 2021*.https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2022.27.33.2200070)

* In our study, nasopharyngeal version had the best results, in  Brümmer et al. it was a nasal version of the same test.

# Results (Delta) – overall

| Number of days between AG-RDT and PCR | Total samples | PCR-positive cases | PCR test positivity in % | Sensitivity in % (95% CI) | Specificity in % (95% CI) | PPV in % | NPV in % |
|---|---|---|---|---|---|---|---|
| PCR up to 3 days after AG-RDT (n = 450 test types) | | | | | | | |
| 0–3 | 346,221 | 68,579 | 19.8 | 72.4 (72.0–72.7) | 96.7 (96.7–96.8) | 84.5 | 93.4 |
| Composite subdatasets (n = 450) | | | | | | | |
| 0 | 86,016 | 15,945 | 18.5 | 80.4 (79.8–81.1) | 96.0 (95.9–96.2) | 82.2 | 95.6 |
| 1 | 140,265 | 31,421 | 22.4 | 80.1 (79.6–80.5) | 95.9 (95.8–96.0) | 84.9 | 94.3 |
| 2 | 60,758 | 12,971 | 21.3 | 64.6 (63.8–65.4) | 97.3 (97.2–97.5) | 86.8 | 91.0 |
| 3 | 59,182 | 8,242 | 13.9 | 39.6 (38.5–40.6) | 98.9 (98.8–99.0) | 85.2 | 91.0 |

Average sensitivity for up to 3 days is most similar to the average sensitivity of **71.2%** (112,323 samples, 61 AG-RDTs) as determined by the metastudy of Brümmer et al, 2021

Same-day AG-RDT and PCRs have the highest sensitivity, which we attribute to higher viral load in persons taking AG-RDT and PCRs on the same day

Data: Aug – Nov 2021, Czechia

# *Omicron vs Delta  - software demo*

# Total number of tests, Omicron variant:

Preliminary

| AG- then PCR- | AG- then PCR+ | AG+ then PCR- | AG+ then PCR+ | PCR+ | PCR- | Total tests | Specificity | Senzitivity |
|---|---|---|---|---|---|---|---|---|
| 53511 | 15306 | 5330 | 42383 | 57689 | 58841 | 116530 | 90,94 % | 73,47 % |

## Symptoms:

Temperature
- no
- yes

Diarrhea / vomiting
- no
- yes

Lost of taste, smell
- no
- yes

Other symptoms
- no
- yes

Cought
- no
- yes

Pain in muscles, joints, chills
- no
- yes

# Presence of AG-RDTs on approved lists

# Results (Delta) – presence on approved lists

| Group of AG RDTs (distinct tests) | Total samples | PCR-positive cases | PCR test positivity in % | Sensitivity in % (95% CI) | Specificity in % (95% CI) |
|---|---|---|---|---|---|
| On WHO EUL 2020 (n = 2*) | 27,528 | 7,053 | 25.6 | 80.2 (79.3–81.2) | 97.0 (96.8–97.3) |
| On WHO EUL 2022 list (n = 3*) | 34,353 | 9,002 | 26.2 | 81.3 (80.5–82.1) | 96.7 (96.5–96.9) |
| On EU Common List (n = 20*) | 144,979 | 30,848 | 21.3 | 74.4 (73.9–74.9) | 97.2 (97.1–97.3) |
| On UK DHSC list (n = 7*) | 45,739 | 10,362 | 22.7 | 74.2 (73.3–75.0) | 97.1 (96.9–97.2) |
| On PEI list – passed sensitivity criteria (n = 20*) | 190,833 | 36,464 | 19.1 | 69.1 (68.6–69.6) | 97.3 (97.2–97.3) |
| On PEI List – passed and on EU Common List (n = 15*) | 130,966 | 27,627 | 21.1 | 74.6 (74.1–75.1) | 97.1 (97.0–97.2) |

PCR up to three days after AG-RDT

Data: Aug – Nov 2021, Czechia

Higher sensitivity was observed for AG-RDTs on approved lists at least partly based on clinical studies

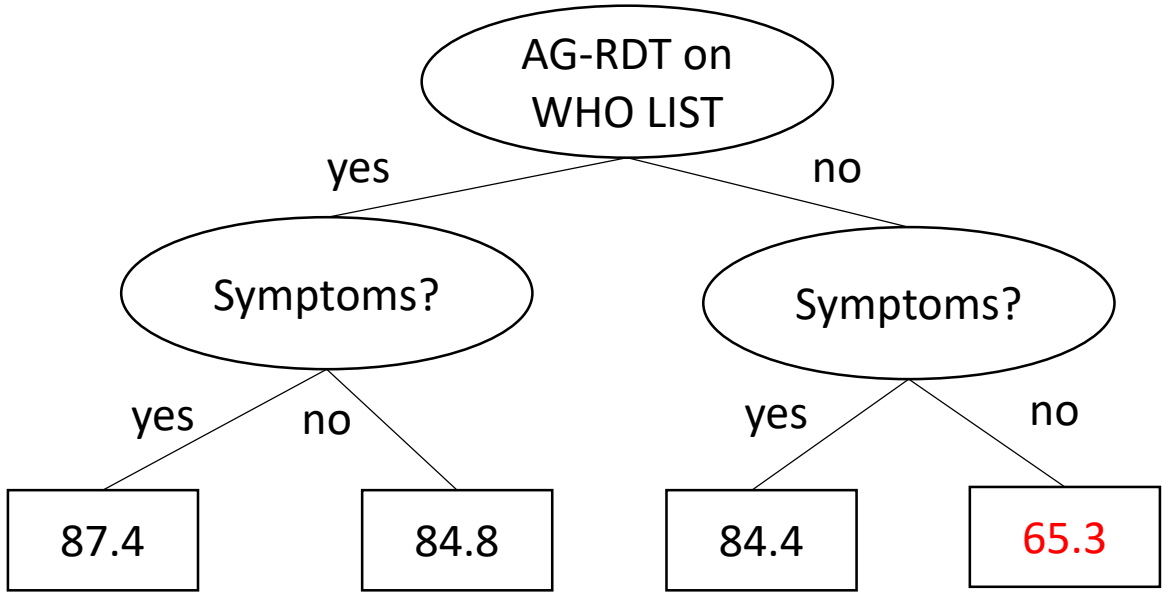
* Although only the most commonly used AG-RDTs in the studied period were included, the number of samples per individual AG-RDTs varies significantly.

Compare with dataset average sensitivity of 72.4%

Results relate to the EU list **from 6 May 2022** (version with only one category of passing tests)

# Results (Delta) – presence on lists and viral load

- Reported symptoms are used as a proxy for higher viral load




Tests not on the WHO list had, on average, lower sensitivity on cases without reported symptoms (i.e. those with lower viral load).

PCR up to three days after AG-RDT

Data: Aug – Nov 2021, Czechia

Preliminary

# Current version of the EU Common list of AG-RDTs

**Category A**: Clinical performance has been evaluated by (at least) one prospective **clinical field study**
**Category B**: Evaluated by a **retrospective in vitro study**.
Studies had to meet the criteria and definitions as agreed by the Health Security Committee on 21 September 2021.

| List | Delta | Omicron |
|---|---|---|
| Sensitivity | | |
| WHO EUL, N=3* AG-RDTs | 79.2 (77.7 to 80.8) N=2,205/2,783 | 75.7 (74.7 to 76.8) N=5,313/7,014 |
| EU Cat A N=28* AG-RDTs | 79.5 (78.2 to 80.7) N=3,513/4,421 | 76.4 (75.6 to 77.2) N=9,109/11,925 |
| EU Cat B N=84* AG-RDTs | 67.8 (65.9 to 69.6), N=1,754/2,588 | 65.0 (64.1 to 65.9), N=7,065/10,865 |
| Specificity | | |
| WHO EUL | 96.7 (96.2 to 97.1) | 95.2 (94.8 to 95.2) |
| Cat A | 96.3 (95.9 to 96.7) | 94.9 (94.5 to 95.2) |
| Cat B | 94.4 (93.8 to 95.0) | 90.6 (90.0 to 91.1) |

Average EU Cat A list sensitivities are comparable to WHO EUL, but the EU list contains more tests

AG-RDTs on the **Category A** part of the list have significantly **higher sensitivity** than AG-RDTs on the Category B of the list

AG-RDTs on the **Category A** part of the list have significantly **higher specificity** than AG-RDTs on the Category B of the list

PCR up to three days after AG-RDT

Delta: 1 Dec 21 – 25 Dec 22, Czechia
Omicron: 20 Jan 22 – 22 Feb 22, Czechia

EU Common list - 22 July 2022,
WHO EUL – 7 June 2022

* AG-RDT count includes all AG-RDT types with at least one PCR-paired sample in at least one of the two studied periods

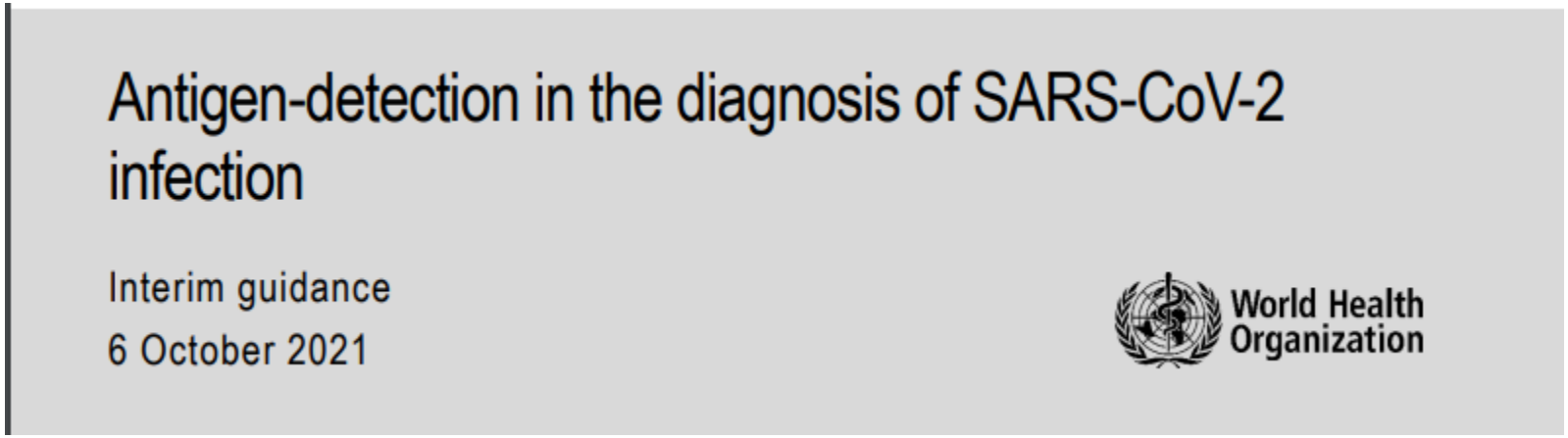
# Field data as a complement to clinical studies in AG-RDT validation?

| **Criterion** |  Independent in-vitro study |  Independent clinical study |   Data registered as part of field use of AG-RDTs and RT-PCR |
|---|---|---|---|
| Used in approved lists | EU List Category B | EU List Category A | Not yet used? |
| Independent | +++: typically done by large public bodies | ++: may be paid for by manufacturer | +++: hundreds of testing sites with no connection to manufacturer |
| Reflects variations between batches | +: one time evaluation | +: one time evaluation | +++: data continuously collected |
| Keeps up with mutations | +: typically fixed pool/panel | ++ | +++: data continuously collected |
| Evaluates sample type | - | +++ | + (+++): not collected in CZ (but could be) |
| Evaluates subgroups (by age, symptoms) | - | +: limited due to sample size | +++: large sample, linkable to other patient data |
| Sensitivity by viral load (Cq) | +++ | +++ | + (+++): Cq values not collected (but could be) |
| Unified methodology across evaluated AG-RDTs | +++ | ++: requirements on EQAP, etc, may vary. | +++: testing sites must conform to uniform state-wide requirements for both AG-RDT and PCR testing |
| Large sample size | +: at least 50 pooled specimen | ++: at least 300 PCR-, 100 PCR+ | ++++: thousands of tests daily |
| Costs | ++ | + | +++: no additional costs, only data processing |
| Other disadvantages compared to clinical studies as gold standard | May not correspond to clinical results | GOLD STANDARD | Subsequent PCR neither done systematically for all AG-RDTs nor randomly (but could be done randomly) |

# Results and conclusions for subpopulations testing

Presented by Dr. Helena Jiřincová
National Reference Laboratory for Influenza and Respiratory Viruses, National Institute of Public Health, CZ

WHO recommends the use of Ag-RDTs that meet minimum performance requirements of **≥ 80% sensitivity** and ≥ 97% specificity.



# 1st Conclusion of the study – AG RDT & children /adolescent field study

- Sensitivity in children (0–12 years) and adolescents (13–18 years) was significantly lower than in adults ($p < 0.05$).

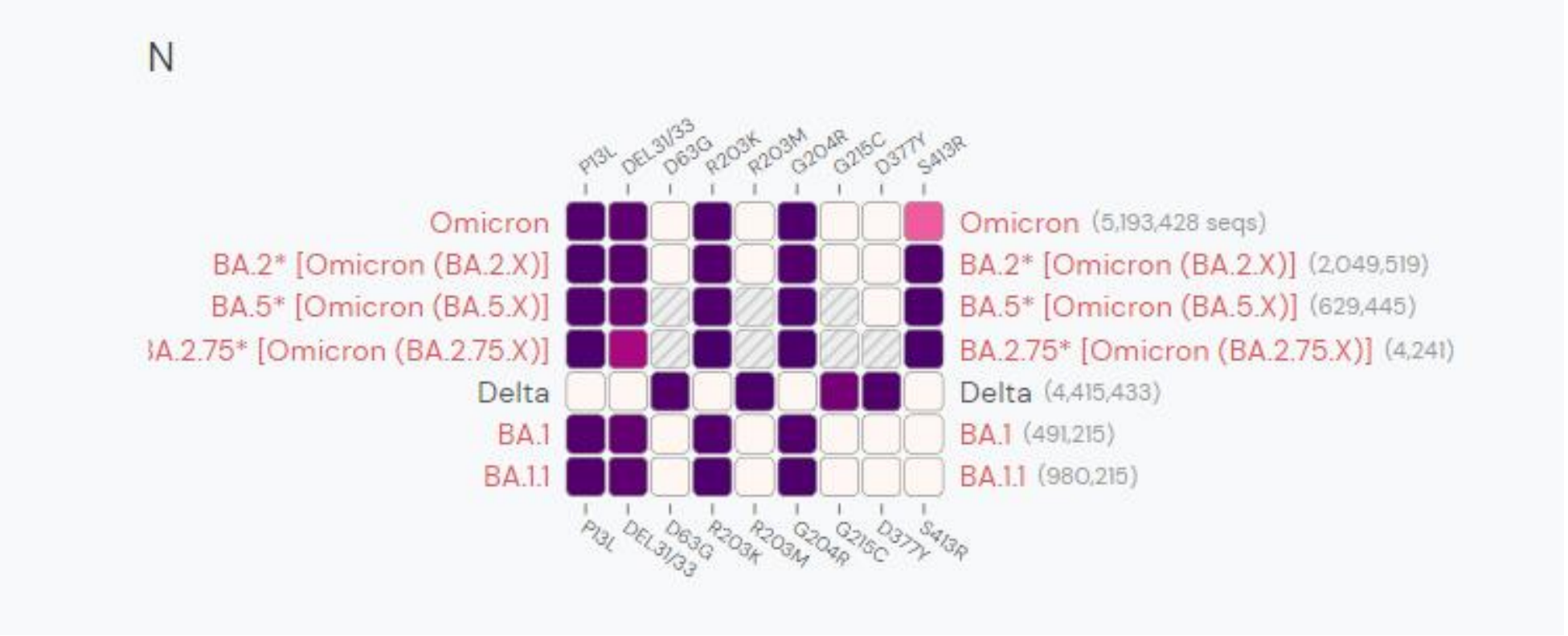
| Group | Total samples | PCR-positive cases | PCR test positivity in % | Sensitivity in % (95% CI) | Specificity in % (95% CI) | PPV in % | NPV in % |
|---|---|---|---|---|---|---|---|
| **Age (years), n = 346,211** | | | | | | | |
| 0–12 | 44,896 | 5,489 | 12.2 | 65.5 (64.2–66.7) | 97.0 (96.8–97.2) | 75.2 | 95.3 |
| 13–18 | 37,693 | 5,101 | 13.5 | 65.3 (64.0–66.6) | 97.2 (97.0–97.4) | 78.6 | 94.7 |
| 19–25 | 37,126 | 6,153 | 16.6 | 71.0 (69.9–72.2) | 97.0 (96.8–97.4) | 82.6 | 94.4 |
| ≥ 26 | 226,496 | 51,835 | 22.9 | 73.9 (73.5–74.3) | 96.5 (96.4–96.6) | 86.3 | 92.6 |

Data: PCR up to three days after AG-RDT, Aug – Nov 2021, Czechia

- **Sensitivity of AG RDT is the decisive factor in performance, especially in younger age groups**
- **The main criterion for field use (diagnostic, mass screening, etc.) should be performance, not cost**

Our preliminary results for Omicron indicate *increased sensitivity of AG-RDTs in children, adolescents and young adults* compared to results for Delta. This is consistent with the observation of higher viral load in these populations for Omicron BA.2.2 (Ao et al., 2022)

# N protein SNP DELTA x OMICRON




www.outbreak info

# 2nd Conclusion of the study - AG RDT versus incidence/indication

- The higher the incidence, the higher the sensitivity and the lower the specificity.
- Significantly higher sensitivity was obtained for the diagnostic indication – typically symptomatic cases

| Group | Total samples | PCR-positive cases | PCR test positivity in % | Sensitivity in % (95% CI) | Specificity in % (95% CI) | PPV in % | NPV in % |
|---|---|---|---|---|---|---|---|
| **SARS-CoV-2 incidence (new cases per 100,000 persons in the preceding 7 days), n = 346,221** | | | | | | | |
| 0–100 | 154,081 | 6,877 | 4.5 | 66.0 (64.8–67.1) | 98.3 (98.3–98.4) | 65.0 | 98.4 |
| 100–500 | 83,114 | 18,682 | 22.5 | 68.8 (68.1–69.4) | 96.5 (96.4–96.7) | 85.2 | 91.4 |
| 500–1,000 | 64,062 | 23,804 | 37.2 | 73.5 (72.9–74.1) | 93.9 (93.7–94.2) | 87.8 | 85.7 |
| 1,000–1,727 | 44,964 | 19,216 | 42.7 | 76.7 (76.1–77.3) | 92.3 (91.9–92.6) | 88.1 | 84.1 |
| **Indication, n = 343,062** | | | | | | | |
| Diagnostic | 45,039 | 22,423 | 49.8 | 86.1 (85.7–86.6) | 91.6 (91.2–92.0) | 91.1 | 87.0 |
| Epidemiological | 71,442 | 12,279 | 17.2 | 63.6 (62.8–64.5) | 96.4 (96.3–96.6) | 78.8 | 92.7 |
| Preventive | 226,581 | 311,61 | 13.8 | 63.6 (63.1–64.2) | 97.5 (97.4–97.6) | 80.3 | 94.4 |

**AG RDT test should be used in the context of mass testing only in periods higher than 500/100 000 and for symptomatic cases.**

Data: PCR up to three days after AG-RDT, Aug – Nov 2021, Czechia

# 3rd Conclusion of the study - AG RDT versus vaccination status

- Sensitivity levels for both vaccinated subgroups (symptomatic and asymptomatic) were also higher than for the corresponding unvaccinated subgroups ($p < 0.05$).

| Group | Total samples | PCR-positive cases | PCR test positivity in % | Sensitivity in % (95% CI) | Specificity in % (95% CI) | PPV in % | NPV in % |
|---|---|---|---|---|---|---|---|
| **Vaccination status, n = 346,221** | | | | | | | |
| Unvaccinated | 235,795 | 42,985 | 18.2 | 70.8 (70.3–71.2) | 96.9 (96.8–96.9) | 83.4 | 93.7 |
| No symptoms | 164,478 | 18,859 | 11.5 | 55.8 (55.1–56.5) | 97.6 (97.5–97.7) | 75.3 | 94.5 |
| At least one symptom | 33,686 | 17,687 | 52.5 | 84.2 (83.7–84.8) | 91.1 (90.6–91.5) | 91.3 | 83.9 |
| Vaccinated | 110,426 | 25,594 | 23.2 | 75.0 (74.5–75.5) | 96.4 (96.3–96.5) | 86.2 | 92.7 |
| No symptoms | 65,223 | 9,746 | 14.9 | 59.7 (58.8–60.7) | 97.5 (97.3–97.6) | 80.5 | 93.2 |
| At least one symptom | 27,223 | 11,982 | 44.0 | 85.6 (85.0–86.2) | 92.9 (92.5–93.3) | 90.4 | 89.1 |

Data: PCR up to three days after AG-RDT, Aug – Nov 2021, Czechia

- While the difference is statistically significant, its magnitude is low. The vaccination status thus does not influence the performance of AG RDTs.

Preliminary results for Omicron show the same pattern.

# 4th Conclusion of the study - AG RDT versus type of clinical material

- The sensitivity depends on the clinical material, the lowest is observed for saliva and the highest for nasopharyngeal (NSP) swabs according to both methods. Nasal swabs are less sensitive than NSP but better than saliva.

| Group of AG-RDTs (distinct tests) | Total samples | PCR-positive cases | PCR test positivity in % | Sensitivity in % (95% CI) | Specificity in % (95% CI) |
|---|---|---|---|---|---|
| Sample type **determined from test name** (from all AG-RDT tests in the analysed dataset), n = 74 tests, n = 6,545 samples | | | | | |
| Saliva (n=36) | 4,016 | 668 | 16.6 | 51.6 | 95.8 |
| Nasal (n=24) | 2,349 | 651 | 27.7 | 73.9 | 97.1 |
| Nasopharyngeal (n=14) | 180 | 70 | 38.9 | 84.3 | 89.1 |

Data: PCR up to three days after AG-RDT, Aug – Nov 2021, Czechia

| Group of AG-RDTs (distinct tests) | Total samples | PCR-positive cases | PCR test positivity in % | Sensitivity in % (95% CI) | Specificity in % (95% CI) |
|---|---|---|---|---|---|
| Sample type determined **from the EU database** for a subset of the most commonly used tests | | | | | |
| Saliva (1) | 2,639 | 266 | 10.1 | 18.4 | 98.5 |
| Nasal swab (7) | 35,313 | 5522 | 15.6 | 58.6 | 97.3 |
| Nasal swab, Nasopharyngeal swab (8) | 67,751 | 16932 | 25.0 | 78.7 | 97.1 |
| Nasopharyngeal swab (5) | 12,914 | 2596 | 20.1 | 79.7 | 97.8 |

**The type of clinical material can strongly influence the performance of AG RDT. Young children, in particular, refuse NSP; this is another reason for careful selection of the most sensitive AG RDT for preschool children screening.**

**Preliminary results for Omicron also indicate lower sensitivity of saliva AG-RDTs**

# Conclusion I – AG RDT and Public Heath recommendation

- AG RDT sensitivity is a decisive factor in performance, especially in
  - Testing children and adolescents
  - Preventive testing in collective facilities (e.g., homes for the elderly) and risk groups of the population
- Public health authorities cannot rely on the manufacturer's declaration of sensitivity and specificity
- An independent validation study must be conducted before the field study population
- According to the data, the *WHO EUL list* and *Category A of the EU Common List* should be considered the gold standard for the selection and recommendation of AG RDTs
- The main criterion for using a field study should be performance, not cost
- The public health authority should publish and regularly update the approved list of AG RDT

# Conclusion II

- AG RDT test should only be used during periods of higher incidence
- AG RDT tests are reliable in testing symptomatic cases
- Vaccination status does not affect AG RDT performance
- Could using only approved Ag RDTs with good performance eliminate the need for confirmation of positive Ag RDTs by PCR testing?
  - Alleviate the strain on PCR testing in periods of high incidence and reduce costs
  - Assumes that the sample is taken by a medical professional as part of a state-controlled network of testing sites

# Public health opinion recommendation by NRL in November 2020 –January 2021

- Only AG RDTs recommended according to the latest update of WHO/ECDC.
- Self-sampling might be preferred in a school setting
- Saliva has a lower viral load (also supported by experience from PCR testing, better use test requiring nasopharyngeal samples)
- Prefer strategy for **high-throughput PCR test than AG RDT**, increase the level of digitalisation, prefer strategy for the PCR testing of local wastewater (WW)
- Exclude AG RDT with high false-positivity rate
- The list of reliable and Ministry of Health-approved AG RDTs should be presented, and only those tests should be used for field population study

# References and acknowledgments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More information about the project: http://www.szu.cz/ecdc-1

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