

# Afprøvning af SARS-CoV-2 Antigen tests for påvisning af nye varianter i DK (Omikron BA.4 og BA.5)

Testing of SARS-CoV-2 rapid antigen tests detection of new variants in DK (Omicron BA.4 and BA.5)

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## Afprøvning af SARS-CoV-2 Antigen tests for påvisning af nye varianter i DK (Omikron BA.4 og BA.5)

SARS-CoV-2 epidemien skifter løbende karakter med ændringer i smittetal og nye varianter. Dette betyder at der er nødvendigt at skifte teststrategi afhængigt af kapacitet og prævalens af infektionen. Det er således forventeligt at brugen af antigen-tests ("Kvik-tests") vil øges i perioder af epidemien med højt testbehov, hvor PCR-kapaciteten ikke er tilstrækkelig. Der er derfor vigtigt at det sikres at antigen-tests der anvendes, både til professionelt brug og til selv-test, kan påvise SARS-CoV-2 varianter der, til en hver tid, flourerer i Danmark. Senest har det været usikkerhed om Omikron-varianterne BA.4 og BA.5, kan påvises med de anvendte antigen-tests.

Formålet med denne afprøvning er at undersøge om de antigen-tests, der er indkøbt af offentlige myndigheder til anvendelse som selvtest i Danmark, kan påvise Omikron varianterne BA.4 og BA.5.

Den skitserede afprøvning, på SSI, er en enkel, hurtig og laboratoriebaseret kvalitativ afprøvning, hvor det vurderes om den enkelte antigen-test kan detektere Omikron varianterne BA.4 og BA.5 (+/-) sammenligneligt med vildtypevirus (oprindelig variant, "Index"). Afprøvningen kan således ikke betragtes som en kvantitativ performance-sammenligning mellem de enkelte kits. Sådanne undersøgelser udføres ved omfattende kliniske studier.

Afprøvningen foretages ved at sammenligne evnen til at påvise de nye varianter (BA.4 og BA.5) med evnen til at påvise vildtype (index) ved anvendelse af fortyndingsrækker af dyrket virus fortyndet i dyrkningsmedium.

Effektiviteten af påvisning af varianter fremgår ift. Vildtype-virus i rapporteringen for afprøvningen af det enkelte testkit. Endvidere vil det fremgå om det vurderes at være tilfredsstillende.

I denne undersøgelse er der kun inkluderet antigen-tests, der er indkøbt af offentlige myndigheder til anvendelse som selvtest i Danmark, og som er rekvireret af SSI fra SFOS.

### Protokol for afprøvning af SARS-CoV-2 Antigen-tests for påvisning af varianter (Delta og Omikron)

#### SARS-CoV-2 isolater:

Vildtype (Index): Strain SARS-CoV-2/hu/DK/SSI-H5 (dyrket af SSI)

Omicron BA.4: Strain R03682 (gave fra Drs Khadija Khan og Alex Sigal, Africa Health Research Institute, Durban, South Africa)

Omicron BA.5.2: Strain CAP002 (gave fra Drs Khadija Khan og Alex Sigal, Africa Health Research Institute, Durban, South Africa)

Alle varianter blev opformeret i VERO E6 celler. Ved cytopatisk effekt i cellekulturerne, blev dyrkningsmediet (supernatanten) nedfrosset. Viruspræparationer blev fremstillet af optøet og centrifugeret supernatant inden udportionering og opbevaring ved -80C.

Alle varianter havde sammenlignelige Ct-værdier målt med E-gene RT-PCR.

På dagen for testning blev portioner af alle tre varianter tørt op og fortyndet i 10-folds fortyndingsserie.

#### Metode til afprøvning af antigen-tests:

Til kalibrering af fortyndinger for hver testkit, blev vildtype-variant fortyndet i fem trin (1:10- 1:100.000).

Da vildtypevirus kun viste positivt signal i de tre første trin, blev disse tre fortyndinger anvendt i duplikat til evaluering af vildtype- og Omikron-varianterne BA.4 og BA.5.

Omikron-varianterne blev således efterfølgende fortyndet i tre trin (1:10 -1:1.000). 100 µL fortyndet virus blev også brugt som prøve i alle testkits.

Fortyndinger af virus blev foretaget i celledyrkningsmedie; Dulbeccos modified Eagle's medie (DMEM). Anvendelse af DMEM som negative fortyndingsmedie blev testet for hvert antigenkit i duplikat, og fundet negativt i alle tests.

### Resultater fra afprøvning af antigen testkits til uge 30 2022:

Name of test	Manufacturer/ supplier	Variant	Dilution of variant			
			1:10	1:100	1:1.000	1:10.000
Flowflex SARS-CoV-2 Antigen	Acon Biotech	Wild-type	+	+	+	-
Rapid Test (self testing)		BA.4	+	+	+	nd
		BA.5	+	+	+	nd
BIOSYNEX COVID-19 Antigen	BIOSYNEX	Wild-type	+	+	-	-
selvtestkit		BA.4	+	+	(+)	nd
		BA.5	+	+	(+)	nd
SARS-COV-2 Antigen Self test Nasal	Roche	Wild-type	+	+	-	-
		BA.4	+	+	-	nd
		BA.5	+	+	-	nd
Rapid SARS-COV-2 Antigen Test Card	Boson Biotech	Wild-type	+	+	+	-
		BA.4	+	+	+	nd
		BA.5	+	+	+	nd

+: positive

(+): weak positive

-: negative

Når afprøvningen viste samme resultat for alle duplikaterne, er kun vist et resultat for hver fortynding/ variant. Forskelligt resultat i duplikaterne er vist med (+)/-.

### Konklusion:

Afprøvningen af de 4 afprøvede antigen-tests viser at alle tests kan påvise både Omikron BA.4 og BA.5 varianterne på sammenligneligt niveau med vildtypen (Index). Forskelle i det generelle niveau for påvisning af varianterne (positive ved forskellige fortyndinger), mellem de forskellige test, kan skyldes forskelle i egnetheden af at anvende et uniformt celledyrkningsmedium til fortynding af virus i afprøvningen. Disse forskelle skal således ikke tolkes som forskelle i ydeevnen af testene til at påvise SARS-CoV-2 i kliniske prøver.

Den overordnede konklusion er at antigen-tests, der er indkøbt af offentlige myndigheder til anvendelse som selvtest i Danmark, kan påvise Omikron varianterne BA.4 og BA.5

## Testing of SARS-CoV-2 rapid antigen tests detection of new variants in DK (Omicron BA.4 and BA.5)

The SARS-CoV-2 epidemic changes repeatedly with the different number of infected persons and with new variants. This means that it is necessary to change test strategy depending on the test capacity and the prevalence of the infection. Hence, it is expected that the use of rapid antigen tests will increase whenever there is a high need or demand for tests, and the PCR capacity is not sufficient.

Therefore, it is important to make sure that the antigen-tests in use – both for professional use and for self-tests – can detect the SARS-CoV-2 variants that at any given time exist in Denmark. Lately, there has been uncertainty about whether the antigen-tests in use can detect the Omicron-variants BA.4 and BA.5.

The goal of this testing is to examine if the antigen-tests which have been purchased by public authorities for use as self-tests in Denmark can detect the new variants of SARS-CoV-2, which exist in Denmark.

The testing at Statens Serum Institut (SSI) is a simple, quick and lab-based qualitative testing, where it is assessed whether an antigen-test can detect the Omicron variants BA.4 and BA.5 (+/-) comparable to the wild-type (the original variant, "Index"). Hence, the testing cannot be considered as a quantitative performance comparison between the different types of antigen-test kits. Such examinations are investigated in comprehensive clinical studies.

The testing is performed by comparing the ability to detect the new variants (BA.4 and BA.5) with the ability to detect the wild-type (index) by the use of dilution series of cultivated virus diluted in cell culture media.

The efficiency of detection of variants will appear in relation to the wild-type virus in the reporting for testing of each test kit. Furthermore, it will appear whether it is assessed to be satisfactory.

This study only includes antigen tests that have been purchased by public authorities for use as self-tests in Denmark, and which have been requested by SSI from SFOS.

## Protocol for testing of SARS-CoV-2 rapid antigen tests for detection of variants (Delta and Omicron)

### SARS-CoV-2 isolates

Vildtype (Index): Strain SARS-CoV-2/hu/DK/SSI-H5 (cultured by SSI)

Omicron BA.4: Strain R03682 (a generous gift from Drs Khadija Khan and Alex Sigal, Africa Health Research Institute, Durban, South Africa).

Omicron BA.5.2: Strain CAP002 (a generous gift from Drs Khadija Khan and Alex Sigal, Africa Health Research Institute, Durban, South Africa).

All variants were cultivated in VERO E6 cells. Upon cytopathic effect, supernatants were frozen. Virus preparations were made from thawed and centrifuged supernatant before aliquoting and storage at -80C. All variants had a similar Ct-value measured using E-gene RT-PCR. Portions of all three variants were on the day of the testing thawed and diluted in a 10-fold dilution series.

### The method of testing of antigen-tests:

The wild-type variant was diluted in five steps (1:10-1:100.000) in the calibration of dilutions for every test kit. Since the wild-type virus only showed positive signals in the three first steps, these three first dilutions were used in duplicate to the assessment of the wild-type variant and the Omikron-variants BA.4 og BA.5.

Hence, the Omicron-variants were subsequently diluted in three steps (1:10 – 1:1.000). 100 µL diluted virus was also used as a sample in all the test kits. Dilutions of the virus were made in the cell culture media; Dulbecco's Modified Eagle's Medium (DMEM).

The use of DMEM as a negative media for dilution was tested for each antigen kit in duplicate, and found negative.

#### Results of testing of antigen test-kits week 30 2022:

Name of test	Manufacturer/ supplier	Variant	Dilution of variant			
			1:10	1:100	1:1.000	1:10.000
Flowflex SARS-CoV-2 Antigen	Acon Biotech	Wild-type	+	+	+	-
Rapid Test (self testing)		BA.4	+	+	+	nd
		BA.5	+	+	+	nd
BIOSYNEX COVID-19 Antigen	BIOSYNEX	Wild-type	+	+	-	-
selvtestkit		BA.4	+	+	(+)	nd
		BA.5	+	+	(+)	nd
SARS-COV-2 Antigen Self test Nasal	Roche	Wild-type	+	+	-	-
		BA.4	+	+	-	nd
		BA.5	+	+	-	nd
Rapid SARS-COV-2 Antigen Test Card	Boson Biotech	Wild-type	+	+	+	-
		BA.4	+	+	+	nd
		BA.5	+	+	+	nd

(+): weak positive

-: negative

nd: not done

When testing showed the same results for all duplicates, the table only shows one result for every dilution/variant. Different results for the duplicates are shown as (+)/-.

#### Conclusion:

The testing of the antigen tests shows that all the tests can detect the BA.4 and BA.5 variants on a comparable level with the wild-type (Index). Differences in the general level of detection of variants (positive with different dilutions) between the different tests can be due to differences in applicability of using a uniform cell culture media to dilute virus in the testing. Therefore, these differences should not be interpreted as differences in the performance of the tests to detect SARS-CoV-2 in clinical tests.

The overall conclusion is that the antigen tests, which have been purchased by public authorities for use as self-tests in Denmark, can detect the variants Delta and Omicron variants BA.4 and BA.5.