

Afprøvning af SARS-CoV-2 Antigen tests for påvisning af varianter (Delta og Omikron)

Testing of SARS-CoV-2 rapid antigen tests detection of variants (Delta and Omicron)

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Afprøvning af SARS-CoV-2 Antigen tests for påvisning af varianter (Delta og Omikron)

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, florerer i Danmark. Senest har det været usikkerhed om Omikron-varianten kan påvises med de anvendte antigen-tests.

Formålet med denne afprøvning er at undersøge om de enkelte antigen-tests, der anvendes, og ønskes anvendt, i Danmark, kan detektere de varianter af SARS-CoV-2 der er til stede i DK.

Den skitserede afprøvning, på SSI, er en enkel, hurtig og laboratoriebaseret kvalitativ afprøvning, hvor det vurderes om den enkelte antigen-test kan detektere Delta og Omikron varianterne (+/-) sammenligneligt med vildtypevirus (oprindelig variant, "Wuhan"). 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 (Delta og Omikron) med evnen til at påvise vildtype 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.

SSI indbyder producenter og importører af antigen-tests til fremsende testkits (100 stk.), der anvendes i Danmark, til afprøvning på SSI. SSI vil efter modtagelse af testkits, relativt hurtigt (ca. 1 uge fra modtagelse af testen), kunne melde ud på SSIs hjemmeside, om en given antigen-test vil være i stand til at påvise en evt. ny variant (for nuværende Omikron). Ved fremtidige nye varianter kræver dette at SSI har isoleret virus, inden afprøvningen kan foretages.

Test-kits kan efter aftale sendes til:

Claus Nielsen
Bygning 85, rum 322
Statens Serum Institut
Artillerivej 5
2300 København S
Mail: cn@ssi.dk
Tlf.: 32683280

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

SARS-CoV-2 isolater:

Wild type: Strain SARS-CoV-2/hu/DK/SSI-H5,
Delta: Strain SARS-CoV-2/hu/DK/SSI-H11,
Omicron: Strain SARS-CoV-2/hu/DK/SSI-H46.

De tre varianter af dyrket SARS-CoV-2 virus, Wuhan-like (vildtype), Delta og Omikron, blev dyrket 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 tøet op og fortyndet i 10-folds fortyndingsserie.

Variant	Isolate
Vildtype	Strain SARS-CoV-2/hu/DK/SSI-H5,
Delta	Strain SARS-CoV-2/hu/DK/SSI-H11,
Omikron	Strain SARS-CoV-2/hu/DK/SSI-H46,

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-, Delta- og Omikron-varianterne.

Delta- og 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 51 2021:

Name of test	Manufacturer/ supplier	Variant	Dilution of variant		
			1:10	1:100	1:1.000
Panbio - COVID-19 Ag RAPID TEST (NASOPHYNGEAL)	Abbott	Wild-type	+	+	-
		Delta	+	+	(+)
		Omikron	+	+	(+)
SARS-COV-2 Rapid Antigen test	Roche	Wild-type	+	+	(+)
		Delta	+	+	(+)
		Omikron	+	+	(+)
Flowflex SARS-CoV-2 Antigen Rapid Test	Acon Biotech	Wild-type	+	+	+
		Delta	+	+	+
		Omikron	+	+	+
Onsite Covid-19 Ag Rapid Test	CTK Biotech	Wild-type	+	(+)	-
		Delta	+	(+)	-
		Omikron	+	(+)	-

Name of test	Manufacturer/ supplier	Variant	Dilution of variant		
			1:10	1:100	1:1.000
SARS-COV-2 Rapid Antigen test Nasal	Roche (Falck)	Wild-type	+	+	(+)
		Delta	+	+	(+)
		Omicron	+	+	(+)
Standard Q, Covid-19 Ag Nasal	SD Biosensor (CPH Med)	Wild-type	+	+	-
		Delta	+	+	(+)
		Omicron	+	+	(+)
BIOSYNEX COVID-19 Ag BSS	BIOSYNTEX (Carelink)	Wild-type	+	+	(+)
		Delta	+	+	(+)
		Omicron	+	+	(+)
SARS-COV-2 Antigen Self test Nasal	Roche	Wild-type	+	+	-
		Delta	+	+	(+)
		Omicron	+	+	(+)
Rapid SARS-COV-2 Antigen Test Card	Boson Biotech	Wild-type	+	+	(+)
		Delta	+	+	(+)
		Omicron	+	+	(+)
Covid-Rapid, SARS-COV-2 Antigen (N/OP/NP)	api Pharma (VINGMED/ Vicare)	Wild-type	+	(+)	-
		Delta	+	(+)	-
		Omicron	+	(+)	-

+: positive

(+): weak positive

-: negative

Afprøvningen viste samme resultat for alle duplikaterne, derfor er der kun vist et resultat for hver fortynding/variant.

Resultater fra afprøvning af antigen testkits til uge 3 2022:

Name of test	Manufacturer/ supplier	Variant	Dilution of variant			
			1:10	1:100	1:1.000	1:10.000
SARS-COV-2 Antigen Rapid test (Self-testing)	HUGHES	Wildtype	+	+	+	-
		Delta	+	+	+	nd
		Omikron	+	+	+	nd
Covid-19 Antigen Detection Kit (Colloidal Gold) Self test	Zhuhai Lituo Biotechnology	Wildtype	+	(+)	-	-
		Delta	+	+	-	nd
		Omikron	+	(+)	-	nd
Covid-19 Antigen Detection Kit - Nasal Swap	NEWGENE Bioengineering	Wildtype	+	(+)	-	-
		Delta	+	+	(+)	nd
		Omikron	+	+	-	nd
One Step Test for SARS-CoV-2 An- tigen (Colloidal Gold) Self test	Getein Biotech	Wildtype	+	+	(+)	-
		Delta	+	+	(+)	nd
		Omikron	+	+	(+)	nd

Name of test	Manufacturer/ supplier	Variant	Dilution of variant			
			1:10	1:100	1:1.000	1:10.000
Rapid COVID-19 Antigen Self-Test	HEALGEN	Wildtype	+	+	-	-
		Delta	+	+	(+)	nd
		Omikron	+	+	-	nd

+: positive

(+): weak positive

-: negative

nd: not done

Afprøvningen viste samme resultat for alle duplikaterne, derfor er der kun vist et resultat for hver fortynding/variant.

Konklusion:

Afprøvningen af de 15 afprøvede antigen-tests viser at alle tests kan påvise både Delta- og Omikron-varianten på sammenligneligt niveau med vildtypen (Wuhan). 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 pt. anvendes i Danmark kan påvise både delta- og omikronvarianterne.

Testing of SARS-CoV-2 rapid antigen tests detection of variants (Delta and Omicron)

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-variant.

The goal of this testing is to examine if the antigen-tests in use in Denmark can detect the 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 Delta and Omicron variant (+/-) comparable to the wild-type (the original variant, “Wuhan”). 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 (Delta and Omicron) with the ability to detect the wild-type 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.

SSI invites manufacturers and suppliers of antigen-tests to send test kits (100 pcs.), which are in use in Denmark, for SSI to test. SSI will, when the test kits have been received, relatively quickly (app. one week from reception of the test) report if the given antigen-test are capable of detecting a new variant (for now Omicron). When future new variants occur, SSI will first be able to test the test kits when SSI has isolated the new type of virus.

Test-kits can by appointment be sent to:

Claus Nielsen
Building 85, room 322
Statens Serum Institut
Artillerivej 5
2300 Copenhagen S
Mail: cn@ssi.dk
Phone: +45 3268 3280

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

SARS-CoV-2 isolates

Wild-type: Strain SARS-CoV-2/hu/DK/SSI-H5,
Delta: Strain SARS-CoV-2/hu/DK/SSI-H11,
Omicron: Strain SARS-CoV-2/hu/DK/SSI-H46.

The three variants of cultivated SARS-CoV-2 virus, Wuhan-like (wild-type), Delta and Omicron, were all 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.

Variant	Isolate
Wild-type	Strain SARS-CoV-2/hu/DK/SSI-H5,
Delta	Strain SARS-CoV-2/hu/DK/SSI-H11,
Omicron	Strain SARS-CoV-2/hu/DK/SSI-H46,

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, Delta variant and Omicron variant.

Hence, the Delta- and 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 51 2021:

Name of test	Manufacturer/ supplier	Variant	Dilution of variant		
			1:10	1:100	1:1.000
Panbio - COVID-19 Ag RAPID TEST (NASOPHYNGEAL)	Abbott	Wild-type	+	+	-
		Delta	+	+	(+)
		Omicron	+	+	(+)
SARS-COV-2 Rapid Antigen test	Roche	Wild-type	+	+	(+)
		Delta	+	+	(+)
		Omicron	+	+	(+)
Flowflex SARS-CoV-2 Antigen Rapid Test	Acon Biotech	Wild-type	+	+	+
		Delta	+	+	+
		Omicron	+	+	+
Onsite Covid-19 Ag Rapid Test	CTK Biotech	Wild-type	+	(+)	-
		Delta	+	(+)	-
		Omicron	+	(+)	-

Name of test	Manufacturer/ supplier	Variant	Dilution of variant		
			1:10	1:100	1:1.000
SARS-COV-2 Rapid Antigen test Nasal	Roche	Wild-type	+	+	(+)
	(Falck)	Delta	+	+	(+)
		Omicron	+	+	(+)
Standard Q, Covid-19 Ag Nasal	SD Biosensor	Wild-type	+	+	-
	(CPH Med)	Delta	+	+	(+)
		Omicron	+	+	(+)
BIOSYNEX COVID-19 Ag BSS	BIOSYNTEX	Wild-type	+	+	(+)
	(Carelink)	Delta	+	+	(+)
		Omicron	+	+	(+)
SARS-COV-2 Antigen Self test Nasal	Roche	Wild-type	+	+	-
		Delta	+	+	(+)
		Omicron	+	+	(+)
Rapid SARS-COV-2 Antigen Test Card	Boson Biotech	Wild-type	+	+	(+)
		Delta	+	+	(+)
		Omicron	+	+	(+)
Covid-Rapid, SARS-COV-2 Antigen	api Pharma	Wild-type	+	(+)	-
(N/OP/NP)	(VINGMED/ Vicare)	Delta	+	(+)	-
		Omicron	+	(+)	-

+: positive

(+): weak positive

-: negative

The testing showed the same results for all duplicates. Therefore, the table only shows one result for every dilution/variant.

Results of testing of antigen test-kits week 3 2022:

Name of test	Manufacturer/ supplier	Variant	Dilution of variant			
			1:10	1:100	1:1.000	1:10.000
SARS-COV-2 Antigen Rapid test	HUGHES	Wildtype	+	+	+	-
(Self-testing)		Delta	+	+	+	nd
		Omikron	+	+	+	nd
Covid-19 Antigen Detection Kit	Zhuhai Lituo	Wildtype	+	(+)	-	-
(Colloidal Gold) Self test	Biotechnology	Delta	+	+	-	nd
		Omikron	+	(+)	-	nd
Covid-19 Antigen Detection Kit -	NEWGENE	Wildtype	+	(+)	-	-
Nasal Swap	Bioengineering	Delta	+	+	(+)	nd
		Omikron	+	+	-	nd
One Step Test for SARS-CoV-2 An- tigen	Getein Biotech	Wildtype	+	+	(+)	-
(Colloidal Gold) Self test		Delta	+	+	(+)	nd
		Omikron	+	+	(+)	nd

Name of test	Manufacturer/ supplier	Variant	Dilution of variant			
			1:10	1:100	1:1.000	1:10.000
Rapid COVID-19 Antigen Self-Test	HEALGEN	Wildtype	+	+	-	-
		Delta	+	+	(+)	nd
		Omikron	+	+	-	nd

+: positive

(+): weak positive

-: negative

nd: not done

Afprøvningen viste samme resultat for alle duplikaterne, derfor er der kun vist et resultat for hver fortynding/variant.

Conclusion:

The testing of the 15 tested antigen tests shows that all tests can detect both Delta and Omicron on a comparable level with the wild-type (Wuhan). 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 are currently in use in Denmark, can detect both variants (Delta and Omicron).