Flowflex® SARS-CoV-2 Antigen Rapid Test

A test for detection of SARS-CoV-2 Ag
manufactured by Acon Biotech Hangzhou Co. Ltd.

Report from the evaluation SKUP/2022/128

organised by SKUP at the request of Acon Biotech Hangzhou Co. Ltd.

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SKUP would like to acknowledge with thanks the professional health care workers who contributed to the practical work with this evaluation at Esbjerg COVID-19 test centre, and Aase Rode at the Clinical Diagnostic Department, Hospital of South West Jutland in Esbjerg, and Farshid Ghazi, Josephine Kristensen and Louis Praeger-Jahnsen that helped with the practical work at Bispebjerg Hospital test clinic and analysing the samples for SARS-CoV-2 at the Department of Clinical Biochemistry in Copenhagen NV.

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Attachments with raw data are included only in the copy to Acon Biotech Hangzhou Co. Ltd.

1. Summary of an evaluation provided by SKUP | Flowflex SARS-CoV-2 Ag Rapid Test

Manufacturer	Acon Biotech Hangzhou Co. Ltd.
Supplier	Acon Biotech Hangzhou Co. Ltd. (requesting company)
Launched in Scandinavia	2020

Aim

To assess the diagnostic performance and user-friendliness of Flowflex SARS-CoV-2 Ag Rapid Test when used under real life conditions by intended users in dedicated COVID-19 test centres.

Examination	**Recommended goals and results**
Overall diagnostic sensitivity	WHO recommends a minimum performance requirement of ≥80 % sensitivity compared to a nucleic acid-amplification test (NAAT) reference assay. **Overall diagnostic sensitivity was not met: 75 % (90 % CI: 68-82 %)***
Overall diagnostic specificity	WHO recommends a minimum performance requirement of ≥97 % specificity compared to a NAAT reference assay. **Overall diagnostic specificity was met: 99,6 % (90 % CI: 98,6-99,9 %)***
User-friendliness	**Quality goal;** a total rating of 'Satisfactory' by SKUP. **The quality goal of user-friendliness was fulfilled**

Background

Measurement system	*In vitro* diagnostic rapid test for qualitative detection of SARS-CoV-2
Intended users	Health care professionals
Sample material	Nasal or nasopharyngeal specimen, of which the first was evaluated by SKUP.

Material and methods

Participants	564 persons exposed to individuals with confirmed SARS-CoV-2 infection, of whom 121 (21 %) tested positive on of the comparison methods.
Comparison method	A real time polymerase chain reaction (RT-PCR) method for detection of SARS-CoV-2 at the Clinical Diagnostic Department at the Hospital of South West Jutland in Esbjerg and the Department of Clinical Biochemistry at Bispebjerg Hospital in Copenhagen NV
Analytical procedure	Subjects exposed to an individual with confirmed SARS-CoV-2 infection were invited to participate in the evaluation. The sampling procedure, performed by trained health care professionals, included one oropharyngeal swab sample for RT-PCR detection and one nasal swab sample from both nostrils, for the Flowflex SARS-CoV-2 Ag Rapid Test. The oropharyngeal swab for RT-PCR detection was immediately placed into sterile tubes, containing 2-3 mL of viral transport media, until transported to the clinical laboratory. The nasal swab was placed into the test vial containing extraction buffer and analysed in accordance with the instructions from the manufacturer. Tree lots of Flowflex SARS-CoV-2 Ag Rapid Test were used.
User-friendliness	Assessed by trained health care professionals using a questionnaire with three given ratings; satisfactory, intermediate and unsatisfactory

Additional results

Sensitivity stratified on cycle threshold (ct) values for the E-gene:	<33: 78 %: (90 % CI: 70-84 %)* <30: 82 %: (90 % CI: 75-88 %)* <25: 83 %: (90 % CI: 76-89 %)*
Prevalence:	21 %
Positive predictive value (PPV):	95 %
Negative predictive value (NPV):	97 %

Acon Biotech Hangzhou Co. Ltd. has accepted the report without further comments

*Confidence interval (CI) is for information only

This summary will also be published in Danish, Norwegian and Swedish at www.skup.org

2. Abbreviations and Acronyms

Ag	Antigen
Ag-RDT	Antigen-detecting Rapid Diagnostic Test
BLS	Biomedical Laboratory Scientist
C-NPU	Committee on Nomenclature, Properties and Units
CI	Confidence Interval
COVID-19	Coronavirus disease 2019
Ct value	Cycle threshold value
DEKS	Danish Institute of External Quality Assurance for Laboratories in the Health Sector
E	Envelope Protein
ECDC	European Centre for Disease Prevention and Control
EQA	External Quality Assessment
Equalis	External quality assessment in laboratory medicine in Sweden
NAATs	Nucleic Acid Amplification Tests
N	Nucleocapsid Protein
Noklus	Norwegian Organization for Quality Improvement of Laboratory Examinations
NPV	Negative Predictive Value
POC	Point of care
PPV	Positive Predictive Value
QCMD	Quality Control for Molecular Diagnostics
RdRP	RNA-dependent RNA polymerase
RNA	Ribonucleic acid
RNP	Human RNase P
RT-PCR	Real Time Polymerase Chain reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SKUP	Scandinavian evaluation of laboratory equipment for point of care testing
WHO	World Health Organization

3. Introduction

The purpose of Scandinavian evaluation of laboratory equipment for point of care testing (SKUP) is to improve the quality of near patient testing in Scandinavia by providing objective information about analytical quality and user-friendliness of laboratory equipment. This information is generated by organising SKUP evaluations in point of care (POC) settings.

3.1. The concept of SKUP evaluations

SKUP evaluations follow common guidelines and the results from various evaluations are comparable[1]. The evaluation set-up and details are described in an evaluation protocol and agreed upon in advance. The analytical results and user-friendliness are assessed according to pre-set quality goals. To fully demonstrate the quality of a product, the end-users should be involved in the evaluation. If possible, SKUP evaluations are carried out using three lot numbers of test cassettes from separate and time-spread productions.

3.2. Background for the evaluation

In December 2019, Wuhan city in Hubei Province, China, became the center of an outbreak of a severe pneumonia, later identified as caused by a novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) [1]. The virus causes coronavirus disease 2019 (COVID-19). Currently, COVID-19 is mainly diagnosed by detection of ribonucleic acid (RNA) from SARS-CoV-2 using nucleic acid amplification tests (NAATs), such as real time polymerase chain reaction (RT-PCR) assays in a sample collected with a swab from the upper airways [2]. RT-PCR is performed in clinical microbiology laboratories, requiring advanced analytical instruments and trained personnel. The ease-of-use and rapid turnaround time of antigen-detecting rapid diagnostic tests (Ag-RDTs) offer decentralized testing that potentially can expand access to testing and decrease delays in diagnosis [3].

The Flowflex SARS-CoV-2 Antigen (Ag) Rapid Test is an in vitro diagnostic POC rapid test for detection of SARS-CoV-2 in nasal and nasopharyngeal specimens. The product is intended for professional use. Flowflex SARS-CoV-2 Ag test is produced by Acon Biotech Hangzhou Co. Ltd. The SARS-CoV-2 Ag test was launched into the Scandinavian market November 2020. This SKUP evaluation was carried out from March 2021 to February 2022 at the request of Acon Biotech Hangzhou Co. Ltd.

3.3. The aim of the evaluation

The aim of the evaluation was to assess the diagnostic performance and user-friendliness of Flowflex SARS-CoV-2 Ag Rapid Test when using nasal swab specimens under real life conditions by intended users at two dedicated COVID-19 testing centres.

[1]SKUP evaluations are under continuous development. In some cases, it may be difficult to compare earlier protocols, results and reports with more recent ones.

3.4. The model for the evaluation of Flowflex SARS-CoV-2 Ag Rapid Test

The evaluation was carried out at two dedicated COVID-19 test centres to evaluate the performance of Flowflex SARS-CoV-2 Ag Rapid Test in the hands of the intended users, see flowchart in figure 1.

The evaluation included:

- Examination of the diagnostic performance (diagnostic sensitivity and specificity) of the Flowflex SARS-CoV-2 Ag Rapid Test using nasal swab specimens.
- Examination of the diagnostic performance related to different clinical subgroups and cycle threshold (ct) values from the RT-PCR results.
- Evaluation of the user-friendliness of the Flowflex SARS-CoV-2 Ag Rapid Test and its manual by the intended users.

In addition, the positive predictive value (PPV) and the negative predictive value (NPV) were calculated.

Subjects exposed to a previously confirmed case of SARS-CoV-2 infection were included within 10 days of exposure e.g., targeted testing of household members or equivalent close contacts. Both symptomatic and asymptomatic participants were included. Household transmission of SARS-CoV-2 is reported to be high [4], and a prevalence of approximately 20 % was expected. Target number of participants was 100 positive results and 100 negative results, but maximum number included was set to 600. For comparison and assessment of the diagnostic sensitivity and specificity, an oropharyngeal sample was analysed on an RT-PCR comparison method. In this evaluation two different RT-PCR comparison methods were used.

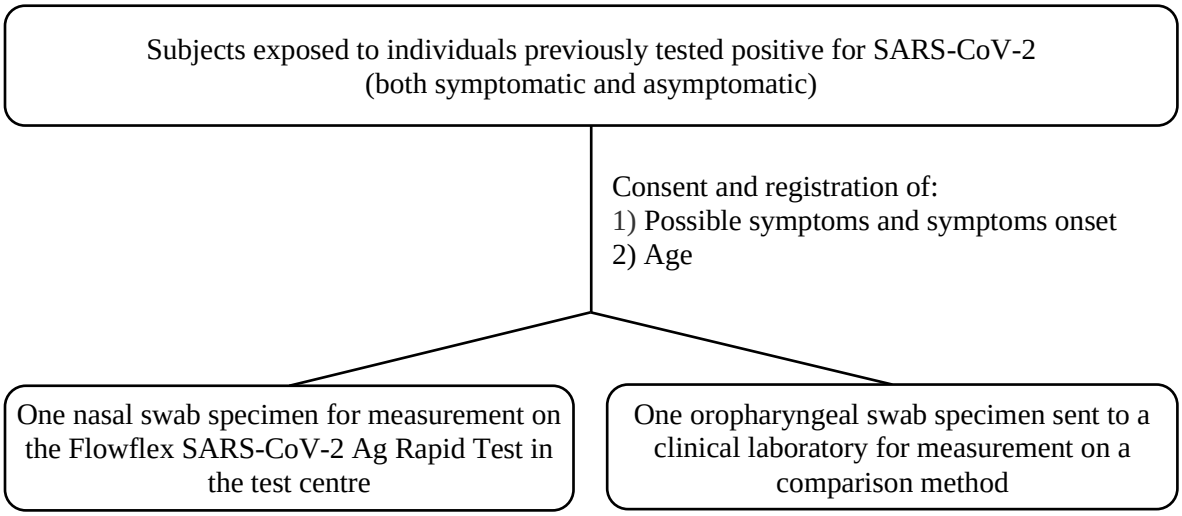

Figure 1. Flowchart illustrating the model of the evaluation. Enrolment of patients was planned to continue until at least 100 positive and at least 100 negative SARS-CoV-2 RT-PCR results were achieved in the clinical laboratory, but maximum number included was set to 600.

4. Quality goals

4.1. Analytical quality

Present recommendations for diagnostic SARS-CoV-2 tests

The World Health Organization (WHO) suggest that SARS-CoV-2 Ag-RDTs that meet the minimum performance requirements of ≥80 % sensitivity and ≥97 % specificity compared to a NAAT reference assay can be used to diagnose SARS-CoV-2 infection, where NAAT is unavailable or where prolonged turnaround times preclude clinical utility. In settings with low prevalence of active SARS-CoV-2 infections specificity should ideally be ≥99 % to avoid many false positive results [3]. The European Centre for Disease Prevention and Control (ECDC) agrees with the minimum performance requirements set by WHO but suggests aiming to use tests with a performance closer to RT-PCR, i.e., ≥90 % sensitivity and ≥97 % specificity [5].

4.2. User-friendliness

The evaluation of user-friendliness was carried out by asking the evaluating personnel in the test centre to fill in a questionnaire, see section 6.4. The tested equipment must reach a total rating of "satisfactory" to fulfil the quality goal.

Technical errors

SKUP recommends that the fraction of tests wasted due to technical errors should not exceed 2 %.

4.3. Principles for the assessments

To qualify for an overall good assessment in a SKUP evaluation, the measuring system must show satisfactory analytical quality as well as satisfactory user-friendliness.

4.3.1. Assessment of the analytical quality

The analytical results are described and discussed related to literature. Statistical expressions and calculations used by SKUP are shown in attachment 5.

Diagnostic sensitivity

The diagnostic sensitivity was calculated as the fraction of the true positive Flowflex SARS-CoV-2 Ag Rapid Test results in proportion to the positive RT-PCR results. The calculated result was given with a 90 % confidence interval (CI) (for information only).

Diagnostic specificity

The diagnostic specificity was calculated as the fraction of the true negative Flowflex SARS-CoV-2 Ag Rapid Test results in proportion to the negative RT-PCR results. The calculated result was given with a 90 % CI (for information only).

Positive and negative predictive values

PPV and NPV were calculated given the prevalence in the tested population and the achieved diagnostic accuracy of the test.

Assessment of three lots
Three lots of test cassettes were used for the purpose of having an evaluation less sensitive to the risk of a poor batch. Separate lot calculations were not performed.

Examination of different clinical subgroups
Sensitivity and specificity were calculated for results stratified on symptoms/no symptoms and days since symptom onset.

Examination of different ct values from the RT-PCR method
The ct value is defined as the number of cycles of amplification required with RT-PCR for the fluorescent signal of the RT-PCR method to reach a threshold above the background signal. The ct value is inversely proportional to the amount of target nucleic acid in the sample (i.e., the lower the ct value the greater the amount of target nucleic acid in the sample). Sensitivity was calculated for positive results stratified on ct values <33, <30 and <25.

4.3.2. Assessment of the user-friendliness

User-friendliness was assessed according to answers and comments given in the questionnaire (see section 6.4). For each question, the evaluator can choose between three given ratings; satisfactory, intermediate and unsatisfactory. To achieve the overall rating "satisfactory", the tested equipment must reach a total rating of "satisfactory" in all four subareas of characteristics described in section 6.4.

Technical errors
The evaluators registered failed measurements and technical errors during the evaluation. The proportion of tests wasted due to technical errors was calculated and taken into account in the assessment of the user-friendliness. User errors related to the handling of the samples were excluded from the calculations.

4.4. SKUP's quality goals in this evaluation

For this evaluation there were no pre-set quality goals for the diagnostic performance of the test. However, SKUP recommends the minimum performance requirements suggested by WHO and the results are discussed related to present literature.

For assessment of the user-friendliness:

User-friendliness, overall rating..... Satisfactory

5. Materials and methods

5.1. Definition of the measurand

The measurement systems are intended to detect SARS-CoV-2 in secrete collected from the upper airways. The Flowflex SARS-CoV-2 Ag Rapid Test detects the presence or absence of the antigens specific for SARS-CoV-2 in nasal and nasopharyngeal specimens. For the comparison method the RNA from SARS-CoV-2 is identified by RT-PCR in oropharyngeal specimens. The results were expressed on an ordinal scale (positive or negative) for both methods. The Committee on Nomenclature, Properties and Units (C-NPU) systematically describes clinical laboratory measurands in a database [6]. The NPU code related to the Flowflex SARS-CoV-2 Ag Rapid Test in this evaluation is NPU59312 (vestibulum nasi). The NPU code related to the comparison method is NPU59178. In this report the term SARS-CoV-2 will be used for the measurand.

5.2. The evaluated measurement system Flowflex SARS-CoV-2 Ag Rapid Test

The information in this section derives from the company's information material.

Flowflex SARS-CoV-2 Ag Rapid Test (figure 2) is a POC test intended for professional use for detection of SARS-CoV-2.

Flowflex SARS-CoV-2 Ag Rapid Test kit includes:

- Flowflex SARS-CoV-2 Ag Rapid Test cassettes
- Sterile nasal swabs
- Pre-filled extraction buffer tubes with dropper caps
- Control swabs (positive and negative)
- Reagent holder
- Package insert

Figure 2. Flowflex SARS-CoV-2 Ag Rapid Test

The Flowflex SARS-CoV-2 Ag Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection of SARS-CoV-2 viral nucleocapsid protein Ag in human nasal and nasopharyngeal specimens.

The test procedure involves collecting nasal or nasopharyngeal specimen, using a recommended swab which is eluted into a tube containing extraction buffer. Four drops of the specimen in extraction buffer are added to the test strip using a dropper cap provided. The test result can be read visually after 15-30 minutes, but not after 30 minutes. Any shade of colour in the test line region (T) of each test cassette should be considered positive.

The formation of a coloured line in the control line region (C) of each test cassette serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

For technical details about the Flowflex SARS-CoV-2 Ag Rapid Test, see table 1. For more information about the Flowflex SARS-CoV-2 Ag Rapid Test system, and name of the manufacturer and the suppliers in the Scandinavian countries, see attachment 2 and 3. For product specifications in this evaluation, see attachment 4.

Table 1. Technical details from the manufacturer

Technical details for Flowflex SARS-CoV-2 Ag Rapid Test	
Sample material	Nasal or nasopharyngeal specimen
Stability of extraction buffer including specimen swab	Specimen should be placed in extraction buffer and tested immediately or within one hour of collection.
Measuring time	15-30 minutes

5.3. The selected comparison method

A selected comparison method is a fully specified method which, in the absence of a Reference method, serves as a common basis for the comparison of the evaluated method.

5.3.1. The selected comparison method in this evaluation

The selected comparison methods in this evaluation were the routine RT-PCR method for SARS-CoV-2 at the Clinical Diagnostic Department, Hospital of South West Jutland in Esbjerg, Denmark and at the Department of Clinical Biochemistry, Bispebjerg Hospital in Copenhagen NV, Denmark hereafter called "comparison methods". The laboratories are accredited according to DS/EN ISO 15189 (2013) by The Danish Accreditation Fund (DANAK). The divisions performing the RT-PCR analysis have 10-20 employees.

Laboratory in Esbjerg	
Method for extraction:	Starlet IVD (Seegene Inc.) STARMag 96 X 4 Viral DNA/RNA Kit
Method for RT-PCR:	Biorad Thermocycler CFX (Bio-Rad Laboratories Inc.) Allplex 2019-nCoV assay
Principle:	RT-PCR detection of the envelope protein (E) gene of the Sarbecovirus, including SARS-CoV-2, the RNA-dependent RNA polymerase (RdRP) gene and nucleocapsid protein (N) gene specific for SARS-CoV-2.
Laboratory in Copenhagen NV	
Method for extraction:	BasePurifier (PentaBase A/S) Nucleo Acid Extraction Kit
Method for RT-PCR:	Biorad Thermocycler CFX (Bio-Rad Laboratories Inc.) CoviDetect FAST assay
Principle:	RT-PCR detection of the E-gene and RdRP-gene of the SARS-CoV-2.

Since the detection of the N-gene is only included for one of the comparison methods in this evaluation, the diagnostic sensitivity of Flowflex SARS-CoV-2 Ag Rapid Test, stratified on ct values are only shown for the E-gene and the RdRP-gene (see section 6.3).

Internal analytical quality control
Allplex 2019-nCoV assay: Kit-dependent positive (mixture of pathogen and IC clones) and negative (RNase-free Water) controls were included in each run. In addition, an internal control (bacteriophage MS2 with RNA) was added to each sample.
CoviDetect FAST assay: Kit-dependent positive and negative controls were included in each run. In addition, an RNP (human RNAse P) internal control was added to each sample.

External analytical quality control
The clinical laboratory in Esbjerg participates in the Quality Control for Molecular Diagnostics (QCMD, United Kingdom) external quality assessment (EQA) scheme for SARS-CoV-2 with five samples in two challenges per year. The clinical laboratory in Copenhagen NV did not participate in a EQA scheme for SARS-CoV-2. The internal control results for the positive samples were instead used to verify the trueness of the comparison method.

5.4. The evaluation

5.4.1. Planning of the evaluation

Inquiry about an evaluation

Acon Biotech Hangzhou Co. Ltd. via Alyssa Lu, International Regulatory Affairs, applied to SKUP in December 2020 for an evaluation of Flowflex SARS-CoV-2 Ag Rapid Test.

Protocol, arrangements and contract

In February 2021, the protocol for the evaluation was approved, and Acon Biotech Hangzhou Co. Ltd. and SKUP signed a contract for the evaluation. A dedicated test centre in Esbjerg and later Bispebjerg Hospital test clinic agreed to represent the intended users in this evaluation. The clinical laboratories in Esbjerg and Copenhagen NV, agreed to perform the respective comparison method measurements.

Training

To optimize performance, WHO recommend that testing with Ag-RDTs should be conducted by trained operators in strict accordance with the manufacturer's instructions. Acon Biotech Hangzhou Co. Ltd. had no local supplier in Denmark, at the time of the evaluation, therefore Acon Hangzhou Co. Ltd. and SKUP agreed that SKUP was responsible for the necessary training of the intended users in both test sites. The training reflected the training usually given to the end-users. Acon Biotech Hangzhou Co. Ltd. was not allowed to contact or supervise the evaluators during the evaluation period.

5.4.2. Evaluation sites and persons involved

At Esbjerg test centre and Bispebjerg Hospital test clinic, 16 and four professional health care workers, respectively, participated in the evaluation. They were all trained before the evaluation in collecting nasal samples from upper airways. They use oropharyngeal swab specimens in the routine work. At the clinical laboratories two biomedical laboratory scientists (BLSs) and one academic worker were involved in the practical work with the comparison methods.

5.4.3. The evaluation procedure

Internal quality control samples were measured on the Flowflex SARS-CoV-2 Ag Rapid Test cassette; the positive control was measured upon opening a new kit and the negative control was measured the same day or next evaluation day.

Recruitment of participants and ethical considerations

Subjects, 16 years or older, exposed to an individual who had previously tested positive for SARS-CoV-2 were asked if they were willing to participate in the evaluation of Flowflex SARS-CoV-2 Ag Rapid Test. Participation was voluntary and verbal informed consent was considered sufficient. Approval from a regional ethical committee was not necessary because the evaluation was considered a quality assurance project.

Handling of the samples and measurement

Test cassettes, buffer and specimens were kept at room temperature (15-30°C) prior to testing.

Nasal swab specimens were used for the measurements on the Flowflex SARS-CoV-2 Ag Rapid Test. In the same sampling session, an oropharyngeal swab specimen was collected for the measurement on the comparison method.

The sampling from each patient was carried out in the following order:

1. Oropharyngeal swab from the throat for the comparison method
2. Nasal swab from both nostrils for the Flowflex SARS-CoV-2 Ag Rapid Test

Nasal swab specimens were collected according to local guidelines and immediately placed into the test vials containing extraction buffer. The extracted samples were measured within one hour of collection, and in accordance with the instructions from the manufacturer. Any shade of colour in the test line region was considered a positive result. In case of technical errors and failed measurements, the test was repeated (by using the same extraction buffer) if possible until a result was obtained. Three lot numbers of test cassettes were used in the evaluation.

Local guidelines for sampling the oropharyngeal swab specimen, for the comparison method, was followed. The swabs for the comparison method were placed immediately into sterile tubes containing 2-3 mL of viral transport media. The tubes were kept at room temperature or at 4°C until transported to the clinical laboratory, where the samples were measured on the comparison method. All samples were treated according to the internal procedures of the laboratory regarding potential interfering substances.

6. Results and discussion

Statistical expressions and calculations used by SKUP are shown in attachment 5.

6.1. Number of samples and study population characteristics

The practical work was performed from spring 2021 to winter 2022. Most of the evaluation took place at the testcenter in Esbjerg, but due to low number of participants tested positive for SARS-CoV-2, the evaluation could not be completed. In November 2021, during the major outbreak of a new COVID-19 variant (Omicron) among the Danish population, the test clinic at Bispebjerg Hospital was recruited to finish the evaluation.

In total 567 participants provided samples for the evaluation and 564 were successfully matched to their corresponding RT-PCR result (table 2). The vast majority were exposed to individuals who had previously tested positive for SARS-CoV-2 and 69 % (n=391) of the participants were in the age-group ≥30 years (table 2). Fifty-four % (n=302) were symptomatic of whom 49 % (n=150) had a symptom duration of ≤5 days, however, 43 % (n=129) of the symptomatic did not state symptom onset. Among those with symptoms, 44 % (n=134) reported two or more symptoms, of which sore throat, headache and dry cough were most commonly reported (not shown). Twenty-one % (n=121) of participants had a positive RT-PCR result. This was higher than the total tested population in Denmark. Investigation among exposed subjects is highly relevant for contact tracing in institutions, semi-closed communities and among household members or equivalent close contacts.

Table 2. Population characteristics

	Total successfully included n (% of all)	**RT-PCR positive results n (% of subgroup)**	**RT-PCR negative results n (% of subgroup)**
Overall	564 (100)	121 (21)	443 (79)
Age			
≤19	39 (7)	6 (15)	33 (85)
20-29	134 (24)	31 (23)	103 (77)
≥30	391 (69)	84 (21)	307 (79)
Symptomatic			
No	262 (46)	31 (12)	231 (88)
Yes	302 (54)	90 (30)	212 (70)
Symptom duration	**n (% of symptomatic)**		
≤5 days	150 (49)	50 (33)	100 (67)
>5 days	23 (8)	9 (39)	14 (61)
Unknown	129 (43)	31 (24)	98 (76)

An account of the number of samples not included in the calculations, is given below.

Missing results

– ID 351, 359 and 360; registration of the Flowflex SARS-CoV-2 Ag Rapid Test result were missing.

Omitted results
There were no omitted results.

Recorded error codes, technical errors and failed measurements
There were no incidences reported that were interpreted as technical errors. Thus, the SKUP recommendation of a fraction of ≤2 % tests wasted caused by technical errors was achieved.

6.2. Analytical quality of the selected comparison method

6.2.1. Internal analytical quality control

All results from the internal analytical quality controls (negative, positive and internal control) were in accordance with the assigned value (data not shown).

6.2.2. The trueness of the comparison method

The trueness of the RT-PCR method for detection of SARS-CoV-2 in the clinical laboratory in Esbjerg was verified with EQA results for the period circumventing the evaluation period (table 3).

Table 3. EQA controls measured on the comparison method at the clinical laboratory in Esbjerg.

Time of measurements	EQA scheme	Sample id	Assigned value (SARS-CoV-2 dPCR Log10 Copies/ml)	Results from the RT-PCR method (ct values; E, RdRP and N-gene)
Week 20	QCMD	SCV2_21C1B-01	Positive (4,13)	Positive (28,4; 28,3 and 27,5)
		SCV2_21C1B-02	Positive (2,51)	Positive (32,9; 32,7 and 32.0)
		SCV2_21C1B-03	Positive (2,00)	Positive (35,8; 38,3 and 34,6)
		SCV2_21C1B-04	Positive (2,94)	Positive (32,5; 32,6 and 31,5)
		SCV2_21C1B-05	Positive (3,15)	Positive (31,5; 32,0 and 30,6)
Week 22	QCMD	SCV2_21C1A-01	Positive (4,10)	Positive (30,0; 30,2 and 29,2)
		SCV2_21C1A-02	Positive (2,94)	Positive (34,0; 34,0 and 33,5)
		SCV2_21C1A-03	Negative (Coronavirus 229E)	Negative
		SCV2_21C1A-04	Positive (3,09)	Positive (33,5; 33,5 and 33,0)
		SCV2_21C1A-05	Positive (2,94)	Positive (34,2; 35,3 and 33,4)

Discussion
The trueness of the comparison method in Esbjerg was confirmed during the evaluation period by the results from the QCMD EQA scheme for SARS-CoV-2. For the comparison method in Copenhagen NV the internal control results of the 82 positive samples were valid, with ct-values <34 [7], in accordance with the instructions from the manufacturer (not shown). Raw data is attached to the requesting company only (attachment 7).

6.3. Analytical quality of Flowflex SARS-CoV-2 Ag Rapid Test

The results below reflect the analytical quality of Flowflex SARS-CoV-2 Ag Rapid Test under real-life conditions in the hand of intended users at a dedicated test centre.

6.3.1. Internal analytical quality control

The results from the internal analytical quality controls (one positive and one negative control) were in accordance with the assigned values (data not shown). Raw data is attached for the requesting company only (attachment 6).

6.3.2. The diagnostic sensitivity of Flowflex SARS-CoV-2 Ag Rapid Test

The diagnostic sensitivity of Flowflex SARS-CoV-2 Ag Rapid Test was calculated as described in attachment 5 using the RT-PCR results as true values, both for the total population, and stratified on clinical subgroups (table 4) and relevant ct values of the target gene detected (table 5 and 6). The calculated results are given with a 90 % confidence interval (CI) (for information only). Raw data is attached for the requesting company only (attachment 7).

Table 4. Diagnostic sensitivity of the Flowflex SARS-CoV-2 Ag Rapid Test measured in nasal specimen. Results achieved by intended users. Overall results and stratified on clinical subgroups.

	Number of positive RT-PCR results	Number of true positive results	Number of false negative results	Diagnostic sensitivity % (90 % CI)
Total	121	91[1]	30[2]	75 (68-82)
Symptomatic				
No	31	20	11	65 (48-79)
Yes	90	71	19	79 (71-86)
≤5 days	50	40	10	80 (68-89)
>5 days	9	7	2	78 (45-96)
Unknown onset	31	24	7	77 (62-89)

[1]Median ct value for the E-gene, true positive results= 16,2 (4,8-32,5). Median ct value for the RdRP-gene, true positive results= 17,2 (6,4-33,5).

[2]Median ct value for the E-gene, false negative results = 23,2 (8,2-35,0). Median ct value for the RdRP-gene, false negative results = 23,9 (9,0-35,7).

Unpaired t test (Excel) p-value <0,001 when comparing the means for the true positive and false negative results for each target gene.

Table 5. Diagnostic sensitivity of Flowflex SARS-CoV-2 Ag Rapid Test measured in nasal specimen. Results achieved by intended users when stratified on ct values for the E-gene.

Ct values	Number of positive RT-PCR results	Number of true positive results	Number of false negative results	Diagnostic sensitivity % (90 % CI)
<33	117	91	26	78 (70-84)
<30	108	89	19	82 (75-88)
<25	95	79	16	83 (76-89)

Table 6. Diagnostic sensitivity of Flowflex SARS-CoV-2 Ag Rapid Test measured in nasal specimen. Results achieved by intended users when stratified on ct values for the RdRP-gene.

Ct values	Number of positive RT-PCR results	Number of true positive results	Number of false negative results	Diagnostic sensitivity % (90 % CI)
<33	114	90	24	79 (72-86)
<30	108	87	21	81 (73-87)
<25	94	78	16	83 (75-89)

6.3.3. The diagnostic specificity of Flowflex SARS-CoV-2 Ag Rapid Test

The diagnostic specificity of Flowflex SARS-CoV-2 Ag Rapid Test was calculated as described in attachment 5 using the RT-PCR results as true values, both for the total population and stratified on clinical subgroups. The calculated results (tables 7) are given with a 90 % CI (for information only). Raw data is attached to the requesting company only (attachment 7).

Table 7. Diagnostic specificity of Flowflex SARS-CoV-2 Ag Rapid Test measured in nasal specimen. Results achieved by intended users. Overall results and stratified on clinical subgroups.

	Number of negative results RT-PCR	Number of true negative results	Number of false positive results	Diagnostic specificity % (90 % CI)
Total	443	441	2	99,6 (98,6-99,9)
Symptomatic				
No	231	230	1	99,6 (98,0-100)
Yes	212	211	1	99,5 (98,0-100)
≤5 days	100	99	1	99,0 (95,3-100)
>5 days	14	14	0	100 (84,8-100)
Unknown onset	98	98	0	100 (97,7-100)

6.3.4. The negative- and positive predictive value of Flowflex SARS-CoV-2 Ag Rapid Test

The PPV was 95 % and NPV was 97 % for the Flowflex SARS-CoV-2 Ag Rapid Test at a prevalence of 21 %. The calculations were performed as described in Attachment 5.

Discussion

The overall diagnostic sensitivity of the Flowflex SARS-CoV-2 Ag Rapid Test was 75 % with a 90 % CI of 68-82 % when compared to the results from the comparison method. PPV was 95 % at prevalence 21 %.

COVID-19 symptoms were reported by 54 % of the participants (table 2). Nearly half of the participant's symptom onset was unknown in this evaluation, but 49 % stated that the symptoms had lasted for five days or less, among these participants the sensitivity was 80 % (table 4). Among the few participants (8 %) that had symptoms lasted for more than 5 days, the sensitivity was 78 %. Participants tested more than 5-7 days since onset of symptoms are more likely to have lower viral loads, and the likelihood of false negative results with Ag-RDTs is higher [3].

For participants without symptoms (46 %), the sensitivity was 65 %, indicating that the test is likely to have lower viral load in asymptomatic than in symptomatic participants although the 90 % CIs were overlapping. This is consistent with findings generally on antigen test performance in asymptomatic individuals [3,8] and emphasises the importance of careful evaluation of the target population before implementing Ag-RDTs for SARS-CoV-2.

The ct values from the comparison method are inversely proportional to the amount of target nucleic acid in the samples measured. The ct value can therefore give some indication of the viral load in the participant. The results stratified on ct values for the respective target gene were similar with overlapping 90 % CIs (table 5 and 6). When only the participants with ct values below 30 were considered, the sensitivity increased to 82 and 81 % (table 5 and 6) for the E-gene and RdRP-gene, respectively. The median ct values for the false negative Flowflex SARS-CoV-2 Ag Rapid Test results were higher than for the true positive results. Of the 30 false negative results, nine had ct values ≥30. Thus, low viral load may have contributed to some of the false negative results. Low viral load suggests that the participants at the time of sampling either were in a pre-symptomatic phase or in a late phase of the infection, and probably non-infectious [9]. From an infection tracing perspective, however, they are still important.

The results stratified by ct values should be interpreted with caution. Due to differences in RT-PCR technology across laboratories, ct values may differ despite equal RNA concentrations in a sample. There is no universal ct value indicating contagiousness. In addition, the viral load in a sample may be affected by preanalytical conditions, e.g., poor sampling can result in different viral loads in samples measured by the Flowflex SARS-CoV-2 Ag Rapid Test and the comparison method even if collected from the same patient at the same time and by the same health care provider. The sampling type between the Flowflex SARS-CoV-2 Ag Rapid Test and the comparison methods may also have affected the viral load.

The overall diagnostic specificity was 99,6 % with a 90 % CI of 98,6-99,9 % (table 7). NPV was 97 % at prevalence 21 %. The main concern when using an Ag-RDTs instead of a RT-PCR method is the risk of false positive results, which is why WHO recommends a higher specificity (≥99 %) for the Ag-RDT tests if used in a low prevalence setting [3]. The risk has been demonstrated in settings with down to 1 % prevalence [10].

Conclusion

In this evaluation, the overall diagnostic sensitivity of Flowflex SARS-CoV-2 Ag Rapid Test did not meet WHO's minimum performance requirement for diagnostic sensitivity (≥80 %), but it did meet the performance requirement for diagnostic specificity (≥97 %) when used under real life-conditions with a prevalence of 21 % by intended users.

6.4. Evaluation of user-friendliness

6.4.1. Questionnaire to the evaluators

The most important responses regarding user-friendliness come from the intended users themselves. The end-users often emphasise other aspects than those pointed out by more extensively trained laboratory personnel.

At the end of the evaluation period, the intended users filled in a questionnaire about the user-friendliness of the measurement system. SKUP has prepared detailed instructions for this.

The questionnaire is divided into four subareas:

Table A) Rating of operation facilities. Is the system easy to handle?
Table B) Rating of the information in the manual / insert / quick guide
Table C) Rating of time factors for the preparation and the measurement
Table D) Rating of performing internal and external analytical quality control

The intended users filled in table A and B. SKUP filled in table C and D and in addition, topics marked with grey colour in table A and B.

In the tables, the first column shows what property is evaluated. The second column in table A and B shows the rating by the users at the evaluation sites (one letter per evaluator). The rest of the columns show the rating options. The overall ratings from all the evaluating sites are marked in coloured and bold text. The total rating is an overall assessment by SKUP of the described property, and not necessarily the arithmetic mean of the rating in the rows. Consequently, a single poor rating can justify an overall poor rating, if this property seriously influences on the user-friendliness of the system.

Unsatisfactory and intermediate ratings are marked with a number and explained below the tables. The intermediate category covers neutral ratings assessed as neither good nor bad.

An assessment of the user-friendliness is subjective, and the topics in the questionnaire may be emphasised differently by different users. The assessment can therefore vary between different persons and between the countries. This will be discussed and taken into account in the overall assessment of the user-friendliness.

Comment
In this evaluation, seven of the 20 professional health care workers (six BLSs and one Laboratory Technician) participated in the user-friendliness of the measurement system by filling out the questionnaire.

Table A. Rating of operation facilities

Topic	Rating	Rating	Rating	Rating	Option
To prepare the test / instrument	**S, S, S, S, S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
To prepare the sample	**S, I[2], S, S, I[2], U[2]**	**Satisfactory**	**Intermediate**	**Unsatisfactory**	No opinion
Application of specimen	**S, I[3], S, S, S, S**	**Satisfactory**	**Intermediate**	Unsatisfactory	No opinion
Specimen volume*	**S, S, S, S, S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Number of procedure step	**S, S, S, S, S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Instrument / test design	**S, S, S, S, S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Reading of the test result	**S, S, S, S, S, I[4]**	**Easy**	**Intermediate**	Difficult	No opinion
Sources of errors	**S, S, S, S, S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Hygiene, when using the test	**S, S, S, S, S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Size and weight of test kit	**S, S, S, S, S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Storage conditions for tests, unopened package	**S**	**+15 to +30°C**	+2 to +8°C	–20°C	
Storage conditions for tests, opened package	**S**	**+15 to +30°C or disposable**	+2 to +8°C	–20°C	
Environmental aspects: waste handling	**S**	**No precautions**	Sorted waste	Special precautions	
Intended users	**S**	**Health care personnel**	Laboratory experience	Biomedical laboratory scientists	
Total rating by SKUP		**Satisfactory**			

* e.g., assessed on whether the volume of extraction buffer was sufficient for repeated measurements.
[1] One person gave intermediate ratings in all topics without an explanation. These ratings are not shown and will not be included in the final assessment because of the risk of bias.
[2] Preparation of the sample would have been easier if the specimen could be collected with an oropharyngeal swab. Comment from SKUP: Nasal or nasopharyngeal samples are the most common for rapid tests of SARS-CoV-2 Ag, and therefore SKUP will not include this rating in the final assessment of the test.
[3] Foam was often generated when dispensing the four drops into the sample well of the cassette.
[4] Some samples resulted in longer waiting time because it was difficult to see if the test result was positive after the 15 minutes.

Table B. Rating of the information in the insert/ quick guide

Topic	Rating[1]	Rating	Rating	Rating	Option
Table of contents/Index	**S, I[2], S, S, S**	**Satisfactory**	**Intermediate**	Unsatisfactory	No opinion
Preparations/Pre-analytic procedure	**S, S, S, S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Specimen collection	**S, I[3], S, S, S**	**Satisfactory**	**Intermediate**	Unsatisfactory	No opinion
Measurement procedure	**S, S, S, S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Reading of result	**S, S, S, S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Description of the sources of error	**S, S, S, S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Help for troubleshooting	**S, S, S, S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Readability / Clarity of presentation	**S, S, S, S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
General impression	**S, S, S, S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Measurement principle	**S**	**Satisfactory**	Intermediate	Unsatisfactory	
Available insert in Danish, Norwegian, Swedish	**S**	**Satisfactory***	Intermediate	Unsatisfactory	
Total rating by SKUP		**Satisfactory**			

* The insert is not available in Norwegian, but in other Scandinavian languages.
[1] Two persons gave intermediate ratings in all topics without an explanation. These ratings are not shown and will not be included in the final assessment because of the risk of bias.
[2] No comment from the evaluator.
[3] The illustrations were small, difficult to see all the details.

Additional negative comments:
Small size text and a lot of text and information.

Table C. Rating of time factors (filled in by SKUP)

Topic	Rating	Rating	Rating
Required training time	**<2 hours**	2 to 8 hours	>8 hours
Durations of preparations/ Pre-analytical time	**<6 min.**	6 to 10 min.	>10 min.
Duration of measurement	**<20 min.**	20 to 30 min.	>30 min.
Stability of test, unopened package	**>5 months**	3 to 5 months	<3 months
Stability of test, opened package	**>30 day or disposable***	14 to 30 days	<14 days
Stability of quality control material, unopened	**>5 months**	3 to 5 months	<3 months
Stability of quality control material, opened	**>6 days or disposable**	2 to 6 days	≤1 day
Total rating by SKUP	**Satisfactory**		

* The test cassette should be used use as soon as possible after opening the foil pouch.

Table D. Rating of analytical quality control (filled in by SKUP)

Topic	Rating	Rating	Rating
Reading of the internal quality control	**Satisfactory**	Intermediate	Unsatisfactory
Usefulness of the internal quality control	**Satisfactory**	Intermediate	Unsatisfactory
External quality control	**Satisfactory**	Intermediate	Unsatisfactory
Total rating by SKUP	**Satisfactory**		

6.4.2. Assessment of the user-friendliness

Assessment of the operation facilities (table A)

The operation facilities were overall assessed as satisfactory, but there were some intermediate and unsatisfactory ratings. The lower ratings mainly concerned the sample material used in the evaluation were different from the one normally used by the evaluation site (not included in the final assessment). In addition, one evaluator mentioned problems with applying the processed specimen to the sampling well due to foam and another complained about the long waiting time until the result could be read.

Assessment of the information in the manual (table B)

The information in the insert was assessed as satisfactory, but there were some intermediate ratings and a negative comment about the small text size and small illustrations.

Assessment of time factors (table C)
The time factors were assessed as satisfactory.

Assessment of analytical quality control possibilities (table D)
The analytical quality control possibilities were assessed as satisfactory.

Conclusion
In all, the user-friendliness of Flowflex SARS-CoV-2 Ag Rapid Test and its manual was rated as satisfactory. The quality goal for user-friendliness was fulfilled.

7. References

1. Chan JF. *et al.* A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* 2020; **395**: 514 – 523.
2. WHO. Recommendations for national SARS-CoV-2 testing strategies and diagnostic capacities, Interim guidance, 25 June 2021, https://www.who.int/publications/i/item/WHO-2019-nCoV-lab-testing-2021.1-eng (accessed 2022-02-21).
3. WHO. Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays, Interim guidance, 11 September 2020, https://www.who.int/publications/i/item/antigen-detection-in-the-diagnosis-of-sars-cov-2infection-using-rapid-immunoassays (accessed 2022-02-21).
4. Madewell ZJ. *et al.* Household Transmission of SARS-CoV-2: A Systematic Review and Meta-analysis. *JAMA Netw Open.* 2020; **3** (12): e2031756.
5. European Centre for Disease Prevention and Control. Options for the use of rapid antigen tests for COVID-19 in the EU/EEA and the UK. 19 November 2020. ECDC: Stockholm; 2020. https://www.ecdc.europa.eu/en/publications-data/options-use-rapid-antigen-tests-covid-19-eueea-first-update (accessed 2022-02-22).
6. The IFCC – IUPAC terminology for properties and units. https://www.ifcc.org/ifcc-scientific-division/sd-committees/c-npu/npusearch/ (accessed 2022-02-22).
7. COVID-19 Multiplex RT-qPCR Assay Instructions for use 2021. https://pentabase.com/wp-content/uploads/2021/05/CoviDetect-FAST-Instructions-for-use-v.1.7_EN.pdf (accessed 2022-02-22)
8. Cochrane Database of Systematic Reviews (Dinnes et al). Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection 2021. https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD013705.pub2 (accessed 2022-02-22)
9. He X. *et al*. Temporal dynamics in viral shedding and transmissibility of COVID-19. *Nat Med* 2020; **26**; 672 – 675.
10. Norwegian Directorate of Health. COVID-19 pandemic: Evaluation of Abbot's Panbio COVID-19 rapid antigen test in Norway, December 2020. https://www.helsedirektoratet.no/rapporter/evaluation-of-abbots-panbio-covid-19-rapid-antigen-test-in-norway/ (accessed 2022-02-22).

Attachments

1. The organisation of SKUP
2. Facts about Flowflex SARS-CoV-2 Ag Rapid Test
3. Information about manufacturer, retailers and marketing
4. Product specifications for this evaluation
5. Statistical expressions and calculations
6. Raw data, internal analytical quality control results, Flowflex SARS-CoV-2 Ag Rapid Test
7. Raw data, Flowflex SARS-CoV-2 Ag Rapid Test and comparison method results

Attachments with raw data are included only in the copy to Acon Biotech Hangzhou Co. Ltd.

The organisation of SKUP

Scandinavian evaluation of laboratory equipment for point of care testing, SKUP, is a co-operative commitment of DEKS[1] in Denmark, Noklus[2] in Norway and Equalis[3] in Sweden. SKUP was established in 1997 at the initiative of laboratory medicine professionals in the three countries. SKUP is led by a Scandinavian *steering committee* and the secretariat is located at Noklus in Bergen, Norway.

The purpose of SKUP is to improve the quality of near patient testing in Scandinavia by providing objective and supplier-independent information about analytical quality and user-friendliness of laboratory equipment. This information is generated by organising SKUP *evaluations*.

SKUP offers manufacturers and suppliers evaluations of laboratory equipment for point of care testing. Provided the equipment is not launched onto the Scandinavian market, it is possible to have a confidential pre-marketing evaluation. The company requesting the evaluation pays the actual testing costs and receives in return an impartial evaluation.

There are *general guidelines* for all SKUP evaluations and for each evaluation a specific *SKUP protocol* is worked out in co-operation with the manufacturer or their representatives. SKUP signs *contracts* with the requesting company and the evaluating laboratories. The analytical results are assessed according to *pre-set quality goals*. To fully demonstrate the quality of a product, the *end-users* should be involved in the evaluations.

Each evaluation is presented in a *SKUP report* to which a unique *report code* is assigned. The code is composed of the acronym SKUP, the year the report was completed and a serial number. A report code, followed by an asterisk (*), indicates an evaluation with a more specific objective. The asterisk is explained on the front page of these protocols and reports.

SKUP reports are published at www.skup.org.

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[1] DEKS (Danish Institute for External Quality Assurance for Laboratories in the Health Sector) is a non-profit organisation owned by the Capital Region of Denmark on behalf of all other Regions in Denmark.

[2] Noklus (Norwegian Organization for Quality Improvement of Laboratory Examinations) is a national not for profit organisation governed by a management committee consisting of representatives from the Norwegian Government, the Norwegian Medical Association and the Norwegian Society of Medical Biochemistry, with the Norwegian Association of Local and Regional Authorities (KS) as observer.

[3] Equalis AB (External quality assessment in laboratory medicine in Sweden) is a limited company in Uppsala, Sweden, owned by “Sveriges Kommuner och Regioner” (Swedish Association of Local Authorities and Regions), “Svenska Läkaresällskapet” (Swedish Society of Medicine) and IBL (Swedish Institute of Biomedical Laboratory Science).

Facts about Flowflex SARS-CoV-2 Ag Rapid Test

This form is filled in by Acon Biotech (Hangzhou) Co. Ltd. in China.

Table 1. Basic facts

Name of the measurement system:	Flowflex SARS-CoV-2 Antigen Rapid Test
Dimensions and weight:	n/a
Components of the measurement system:	Flowflex SARS-CoV-2 Antigen test cassettes, extraction buffer tubes and disposable swabs
Measurand:	Qualitative detection of the nucleocapsid protein antigen to SARS-CoV-2
Sample material:	Nasal and nasopharyngeal swab specimen
Sample volume:	Four drops
Measuring principle:	Lateral flow chromatographic immunoassay
Traceability:	n/a
Calibration:	n/a
Measuring range:	Qualitative
Haematocrit range:	n/a
Measurement time:	15-30 minutes
Operating conditions:	15-30°C, placed on a flat and clean surface
Electrical power supply:	n/a
Recommended regular maintenance:	n/a
Package contents:	The Flowflex SARS-CoV-2 Antigen test kit include: Test Cassettes: 25 tests Extraction Buffer Tubes: 25 tests Positive Control Swab: 1 pcs Negative Control Swab: 1 pcs Disposable Swabs: 25 pcs Package Insert
Necessary equipment not included in the package:	Personal Protective Equipment and Timer

Table 2. Post analytical traceability

Is input of patient identification possible?	n/a
Is input of operator identification possible?	n/a
Can the instrument be connected to a bar-code reader?	n/a

Can the instrument be connected to a printer?	n/a
What can be printed?	n/a
Can the instrument be connected to a PC?	n/a
Can the instrument communicate with LIS (Laboratory Information System)? If yes, is the communication bidirectional?	n/a
What is the storage capacity of the instrument and what is stored in the instrument?	n/a
Is it possible to trace/search for measurement results?	n/a

Table 3. Facts about the reagent/test strips/test cassettes

Name of the reagent/test strips/test cassettes:	Flowflex SARS-CoV-2 Antigen Rapid Test
Stability in unopened sealed vial:	24 months (until expiration date) if stored at temperatures between 2-30 °C
Stability in opened vial:	After removing the test cassette from the foil pouch, it should be used immediately
Package contents:	The test cassettes and extraction buffer are packed individually. Test Cassettes: 25 tests Extraction Buffer Tubes: 25 tests Positive Control Swab: 1 pcs Negative Control Swab: 1 pcs Disposable Swabs: 25 pcs Package Insert

Table 4. Quality control

Electronic self check:	n/a
Recommended control materials and volume:	Positive Control Swab and Negative Control Swab are measured as patient samples
Stability in unopened sealed vial:	24 months (until expiration date) if stored at temperatures between 2-30 °C
Stability in opened vial:	After removing the Control Swab from the foil pouch, it should be used immediately
Package contents:	Included in the Flowflex SARS-CoV-2 Antigen Rapid Test Kit

Information about manufacturer, retailers and marketing

This form is filled in by Acon Biotech (Hangzhou) Co. Ltd.

Table 1. Marketing information

Manufacturer:	Acon Biotech (Hangzhou) Co., Ltd.
Retailers in Scandinavia:	No retailers in Scandinavia
In which countries is the system marketed:	Globally ■ Scandinavia ■ Europe ■
Date for start of marketing the system in Scandinavia:	The product was market in Sweden November 2020.
Date for CE-marking:	September 28, 2020
In which Scandinavian languages is the manual available:	English, Danish, Swedish

Product specifications for this evaluation

Flowflex SARS-CoV-2 Ag Rapid Test cassettes, REF. L031-11815

Lot name in evaluation	Lot no	Expiry date
a	COV2101105R	2023-01-04
b	COV2101118R	2023-01-17
c	COV2101126R	2023-01-25

Flowflex SARS-CoV-2 Ag Rapid Test control swabs, REF. LCD4671-02 / LCD4670-02

Control kit	Lot no	Expiry date
Control swabs, negative and positive (included in the test kit)	COV2101105R COV2101118R COV2101126R	2023-01-04 2023-01-17 2023-01-25

Other equipment used in the evaluation

Equipment	Lot no	Expiry date
Nasal specimen swab (included in the test kit) REF. CF-150-P3B	20201010JZ	2023-10-09
Extraction buffer (included in the test kit) REF. LCD4687-01	COV2101105R COV2101118R COV2101126R	2023-01-04 2023-01-17 2023-01-25

Statistical expressions and calculations

This attachment is valid for evaluations of qualitative test methods with results on the ordinal scale.

Statistical terms and expressions

The definitions and formulas in this section originate from the Geigy document [a].

Statistical calculations

Diagnostic sensitivity is true positive/(true positive + false negative)

Diagnostic specificity is true negative/(false positive + true negative)

Positive predictive value (PPV) is true positive/(true positive + false positive)

Negative predictive value (NPV) is true negative/(true negative + false negative)

Prevalence is true positive/(true positive + true negative + false positive + false negative)

See table 1 for an illustration.

Table 1. Illustration of statistical calculations

	Truth		
	Positive	**Negative**	
Evaluated test positive	a	b	PPV = a/(a+b)
Evaluated test negative	c	d	NPV = d/(d+c)
	Diagnostic sensitivity = a/(a+c)	Diagnostic specificity = d/(b+d)	

Calculation of confidence intervals

Estimation of CI for fractions/proportions is performed according to Adjusted Walds [b]. The CIs are given for information only.

Relationship between PPV / NPV and prevalence
Contrary to diagnostic sensitivity and specificity, the PPV and NPV are related to the prevalence of the disease in a specific population. PPV and NPV are also related to the diagnostic sensitivity and specificity of a diagnostic test.

a. Documenta Geigy. Mathematics and statistics. CIBA-GEIGY Limited, Basel, Switzerland 1971; p 186 formula # 772.
b. https://measuringu.com/calculators/wald/ (accessed 2021-08-04).

Raw data, internal analytical quality control results, Flowflex SARS-CoV-2 Ag Rapid Test

Raw data are included only in the copy to Acon Biotech Hangzhou Co. Ltd.

Raw data, Flowflex SARS-CoV-2 Ag Rapid Test and comparison method results

Raw data are included only in the copy to Acon Biotech Hangzhou Co. Ltd.