Validation Process

QUBA

Testing for life

J. Jongsma
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Van Wees Innovations
Attn. Mr. P. van Wees
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Eurofins Clinical Diagnostics
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Deventer: Februari 2022

Ref. number: EF-VWJJ22

Subject: Validation QUBA breath analyser

Dear mister van Wees,

Please find attached the document concering the validation of the Quick Breath Analizer QUBA COVID-19 analizer.

The evaluation study of the PCR data versus the QuBA breath analyser in this report, incluiding the test details, was done by Prof. Dr. S.A. Morré.

Yours faithfully,
Eurofins

Naam: Johan Jongsma
Functie: Projectleader COVID-19

Evaluation study of the Quick Breath Analyzer; QuBA

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Purpose of the study

The objective of this performance study is to establish the diagnostic sensitivity and specificity of the Quick Breath Analyzer (QuBA) and to provide data to demonstrate that the product is effective in detecting SARS-COV-2 for its intended professional use among symptomatic and asymptomatic persons. The QuBA is compared with the RT-PCR test being the Golden Standard.

Product information

Manufacturer	Van Wees Innovations Europe BV Ambonplein 64 1094 RA Amsterdam The Netherlands
Test name	Quick Breath Analyzer (QuBA)
Detection Method	The QUBA (Quick Breath Analyzer) is a NON-INVASIVE RAPID TEST based on measuring particles of a specific size out of a breath sample which are released after shouting or singing, coming from the tested person, who is in the QuBA cabin.
Intended use	Intended for the qualitative detection of SARS-CoV-2 virus particles in human breath samples
Specimen	Collected virus particles
Content of the test	• A hermetically sealed test cabin with a front door and back door (in and out). • Air filtering device • Particle sizer and counter (SMPS 3910 Nanoscan TSI)
Storage conditions	2 – 40 °C
EC Representative	Van Wees Innovations Europe BV Ambonplein 64 1094 RA Amsterdam The Netherlands CE: NL-CA002-2021-62055

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Test description

The QUBA (Quick Breath Analyzer) is a NON-INVASIVE RAPID TEST based on measuring particles which are released after shouting or singing coming from the tested person.
It's a hermetically sealed test cabin with a front door and back door (in and out). Because the air in the cabin is 99,99997% cleaned before the breath sample is taken, the detection of virus particles is very accurate.
The test procedure takes 150 seconds, in which the test person has to shout or sing for 10 seconds, to exhale as much breath as possible. After 150 seconds the test result is available.

For extensive description of the test see Appendix 4.

Study management

Study coordination
Eurofins Clinical Diagnostics, Munsterstraat 9, 7418 EV Deventer the Netherlands is accredited by the Dutch CDC (RIVM, The Netherlands) for performing SARS-CoV-2 RT-PCR on swabs and Saliva and is ISO 15189 accredited. The study was requested by Van Wees Innovations Europe BV by The CEO Peter W. van Wees (petervanwees@vanweesinnovations.com).

Timeline
The two prospective studies were performed on 4 different locations in December 2020 and December 2021. All these locations were ISO certified and SARS-COV-2 accredited by the Dutch and German government.

Study design

Tests

- RT-PCR tests (NP) were used as reference test (RIVM accorded).
- The QuBA was the test to be evaluated

Expected results

- The PCR is the golden standard and should be the most sensitive and specific assay
- The QuBA is based on a breath sample and expected to have the same or better sensitivity as compared to PCR. This because the measurement is taken directly from the lungs, before the virus settles in the nose or throat.

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Samples

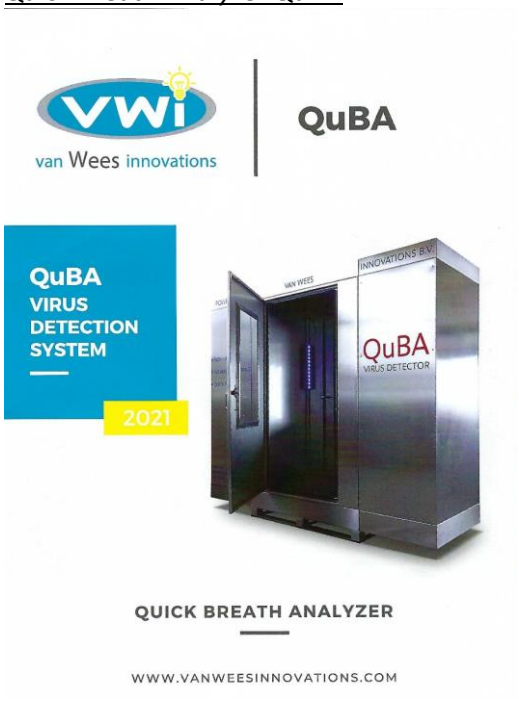
- The study cohort contained both persons with (<7 days) as without Corona-based symptoms, from whom a Naso-/oropharyngeal swab was taken for PCR and a breath sample was taken for the QuBA test for virus particle detection. Both tests were taken by a trained professional.
- 93 SARS CoV-2 RT-PCR positive samples. Sample details were collected including symptoms (<7 days) and absence of symptoms
- 146 SARS CoV-2 RT-PCR negative samples. Sample details were collected including symptoms and absence of symptoms.
- Samples from Client/patiënt positive in the Quba and negative in the PCR are either false positive as compared to PCR or the Quba is more sensitive: for this scenario we asked clients/patients to participate in a follow-up study to detect PCR positivity a second time.

  Appendix 1 for further explanation

Analytical investigation

The Quick Breath Analyzer QuBA sample handling was performed following the instructions of use.
PCR tests were performed used following the instructions of use by trained lab personnel.

Quick Breath Analyzer QuBA



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Definitions:
True positive samples: Sample positive by PCR and QuBA
False positive sample: Sample negative by PCR and positive by QuBA
True negative samples: Sample negative by PCR and QuBA
False negative samples: Sample positive by PCR and negative by QuBA
Specificity (%): # true negative samples / (# true negative samples + # false positive samples) * 100%
Sensitivity (%): # true positive samples / (# true positive samples + # false negative samples) * 100%
Concordance (%): # true positive samples from assure / (#true positive samples Test-X) *100%

Alternative definitions in case the Quba is more sensitive than PCR

Alternative calculation is based on the fact that the QuBA has produced true positives. Therefore the sensitivity of the PCR test will be less than a 100%.

The test procedure

Before entering the QuBA cabin, people have already undergone a PCR swab after which they are asked to fill out a form containing the questions;

1. Male or Female, age
2. Health complaints yes or no
3. PCR batch number for comparison

This form is placed above the TSI SMPS device in order to make a picture from the zero measurement and the final measurement so that the original form is documented together with the results.
People are explained how the test works and can enter the cabin. The operator is the only one that touches the door, entering and exiting the cabin.

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Analysis of QuBA measurements.

The positive / negative decision is made based on the image on the SMPS device in the QuBA.
If there is a significant normal distribution diagram between 45 and 78 nanometers the person is called positive (Image 2).

A negative result comes from the fact that no normal distribution diagram shows between 45 and 78 nanometers, on the SMPS meter.

Image 1: baseline measurement QUBA before the test (negative)

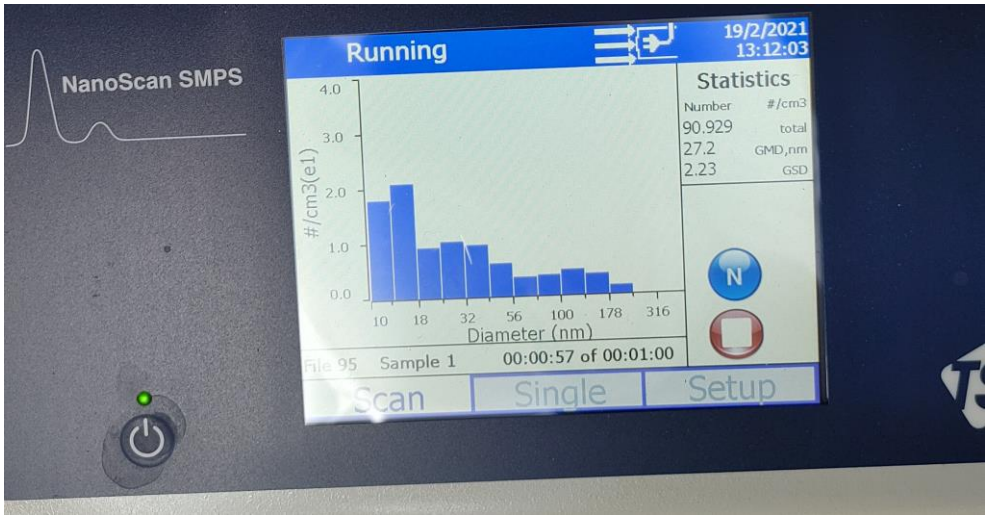

Image 2: baseline measurement QUBA before the test (positive)

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Analytical results

Cohort details

	Total sample taken
N	239
Asymptomatic (n/N) (%)	165/239 (69%)
Symptomatic (n/N) (%)	74/239 (31%)
PCR-positive (n/N) (%)	93/239 (39%)
PCR-positive Asymptomatic (n/N)(%)	54/239 (23%)
PCR-positive Symptomatic (n/N) (%)	39/239 (16%)
PCR Negative (n/N) (%)	146/239 (61%)
PCR sample type	NP

As described in Appendix 1, 4 test persons came back after being tested negative on PCR and positive on QuBA. They all tested positive, this second time on PCR test within 3 days after the first PCR test which was negative.

It concerns the following rows in the spreadsheet in appendix 3;
Y012 Ct value 16,98
Y016 Ct value 26,76
Y129 Ct value 21,57
Y137 Ct value 26,67

Results

In total 239 samples were tested with RT-PCR and the Quick Breath Analyzer.

Sensitivity and specificity QuBA when PCR is Golden standard PCR;

Sensitivity QuBA when PCR is Golden Standard
True pos PCR/(true pos PCR + false neg QuBA) *100%=
93/(93+0)*100% = **100% QuBA**

Specificity QuBA when PCR is Golden Standard
True neg PCR/(true neg PCR+false pos QuBA)
146/(146 +15) *100% = **91% QuBA**

Sensitivity and specificity PCR when true positives are taken from QuBA results instead of PCR results;

Sensitivity PCR True pos QuBA/(true pos QuBA + false neg PCR) =
108/(108+4)= 96,4 **% Sensitivity PCR**

Specificity PCR True neg QuBA/(true neg QuBA + false pos PCR)=
131/(131+0)= 100%

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Results overall tests

Symptomatic patients (mild to moderate complaints)

Tested samples	PCR negative	QUBA negative	PCR positive	QUBA positive
74	35	31	39	43

A-symptomatic patients (no complaints before testing)

Tested samples	PCR negative	QUBA negative	PCR positive	QUBA positive
165	111	100	54	65

As shown in the table above, all PCR positives were also found by the QuBA, resulting in a 100% concordance.

The overall sensitivity of the QuBA as compared to PCR is 100%, independent of the Ct cut-off value used and if the persons had symptoms or not. See appendix 3. The specificity is between 93 % and 100%,, having 93% chance being above 97%. The accuracy is 99,9%. See appendix 2.

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Conclusions

The specificity and sensitivity of the Quick Breath Analyzer (QuBA) by Van Wees Innovations Europe BV was evaluated in this study with 239 samples collected. The QuBA scored very good on the sensitivity (100%).

Sensitivity and specificity QuBA when PCR is Golden standard PCR;

Sensitivity QuBA when PCR is Golden Standard
True pos PCR/(true pos PCR + false neg QuBA) *100%=
93/(93+0)*100% = **100% QuBA**

Specificity QuBA when PCR is Golden Standard
True neg PCR/(true neg PCR+false pos QuBA)
146/(146 +11) *100% = **93% QuBA ***
This is the worst case proven scenario for QuBA

***=** is it plausible to assume that out of the remaining 11 false positives QuBA, there will be at least 5 extra positives? We were unable to retest these 11 people because of privacy legislation. Mathematicallly the chance of getting at least 9 total positives QuBA (out of 15 original false positives QuBA) is 93%. Resulting in a specificity QuBA of at least 97%.

146/(146+0)*100% = **100% Specificity for QuBA**

Sensitivity and specificity PCR when true positives are taken from QuBA results instead of PCR results;

Sensitivity PCR
True pos QuBA/(true pos QuBA + false neg PCR) =
108/(108+4) = 96**% Sensitivity PCR**
This is the best case proven scenario for PCR

108/(108+15) = **88% Sensitivity PCR**
This is the worst case proven scenario for PCR

Specificity PCR
True neg QuBA/(true neg QuBA + false pos PCR)=
131/(131+0)= **100% Specificity PCR**
The differences in test characteristics between persons with-, and without symptoms, is statistically none significant, meaning the QuBA is useful for its intended use.

In conclusion, the results from this study confirm that the Quick Breath Analyzer (QuBA) by Van Wees Innovations Europe BV can be used for the qualitative detection of the airborne virus exhaled by humans, SARS-CoV-2, in breath samples.

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Discussion

The QuBA testing device is very fast and accurate Another great advantage is the device being non-invasive which makes the willingness of people to be tested a lot greater. In addition, the use of the QuBA will not produce any waste material, even the used filters are collected and recycled every 36 months. Given the accuracy and sensitivity the QuBA is suitable for permanent testing facilities. In case of appearance of a new dominant virus, e.g. Rhino, Entero or Influenza, the QuBA can be adjusted, tested and adapted to the detection of such viruses.
For diagnostic reasons, any suspicious test can be confirmed by a PCR test. In case of a negative PCR result, the PCR must be repeated after 4 days.

Second assesor

Evaluation study Quick Breath Analyzer (QuBA)

Prof.dr. Servaas A. Morré
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Hoogleraar Biotechnology and Immunogenetics (SHUATS, India)
Medische Microbiologisch Onderzoeker (MMO)
Biochemicus, Moleculair Bioloog, Immunogeneticus
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Date: 2nd Feb 2022

February 2022
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Appendix 1

In the test in December 2021 there were 10 people tested negative on PCR tests who got a positive test result by the QuBA. In Germany the test team is not allowed to ask the patients to come back for a new PCR test so it's not clear whether the PCR test was not sensitive enough or the QuBA was more sensitive. However, 4 Patients came back later within 3 days after the first test and tested positive with the PCR test. This means that the sensitivity of the QuBA is probably higher than the PCR test.
During the test in December 2020, there was also a difference in positive test results between the PCR and the QuBA. Like the later test, the QuBA tested 5 more people positive then the PCR.
It is likely to assume that in this group of 5 there were also false negatives from the PCR.

Based on these data, we conclude that the QuBA is more sensitive as compared to PCR.

The difference between the positive results in the a- symptomatic patients comes from the fact that the QuBA measures probably contagiousness earlier than a PCR test.
(Confirmation comes, among others, from written statements from clients).

Most likely, the ultimate reliability / accuracy of the tests performed is higher because the QUBA does not assess old infections as positive and probably identifies incipient infections earlier than the PCR method. This needs to be investigated in future longitudinal studies

The link hereunder to the website from RIVM explains why false negatives occur with PCR testing.

https://www.rivm.nl/coronavirus-covid-19/testen/pcr-test#:~:text=Als%20je%20heel%20vroeg%20na,van%20je%20neus%20en%20keel

Foutmarge bij testen

De PCR-test heeft een heel hoge gevoeligheid en geldt internationaal als de 'gouden standaard'. Dat wil zeggen dat dit als de beste test beschouwd wordt en gebruikt wordt om de betrouwbaarheid van nieuwe testen te onderzoeken. Elke test en dus ook de PCR-test heeft altijd een percentage fout-positieven (iemand is niet besmet, maar volgens de test is dat wel het geval) en fout-negatieven (de patiënt is besmet, maar de test geeft dat niet aan). Daarvoor is een aantal mogelijke oorzaken:

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- Als je heel vroeg na een besmetting test, kan de test nog negatief zijn (de test geeft dus aan dat je het virus niet bij je draagt). Dit komt omdat het enkele dagen tot meer dan een week kan duren voordat er voor de test voldoende virus aanwezig is in de cellen van je neus en keel. Er is dan nog niet genoeg virus om met de test te vinden.
- Als je te laat test, als iemand bijvoorbeeld al meer dan een week klachten heeft en al goed is opgeknapt, kan het zijn dat het virus niet meer voldoende aanwezig is in de neus en keel. In dat geval is er weinig virus meer aanwezig en is er ook weinig kans dat iemand nog anderen zal besmetten.
- Aan de andere kant is het mogelijk dat er nog meerdere weken na de infectie stukjes virus in de keel aanwezig blijven, zonder dat er nog besmettelijke virusdeeltjes zijn. Dan kan iemand nog wel positief testen, maar is de persoon niet meer besmettelijk voor anderen.
- Een test kan ook ten onrechte de uitslag geven dat je het coronavirus niet hebt als met de wattenstok niet goed genoeg slijm van de neus en keel is afgenomen, of omdat niet de juiste soort wattenstok is gebruikt of omdat niet op de goede plek in de neus en keel slijm is afgenomen
- Sowieso heeft elke test een fout marge, ook de PCR. De kans op een fout-positieve uitslag is bij een PCR heel klein. De kans op een positieve testuitslag terwijl de persoon niet besmet of besmettelijk is, is vooral aanwezig bij een laag en dalend aantal infecties in een land of als je mensen gaat testen die geen klachten hebben.

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Appendix 2
Subject: Reliability of the QuBA.

The test results of the QuBA depend on the following three points;

1. There must be a dominant virus,
2. It must spread through aerosols and
3. The diameter must be known.

Because there is a dominant virus, we can attribute any test results to this dominant virus. In addition, because we have a broad scope from 10 nanometers to 420 nanometers, the chance of an incorrect diagnosis will decrease enormously. The equipment used is the Nanoscan 3910 from TSI. This device not only scans the diameter of the particles but also measures the numbers in the various size sections.
Because the virus continues to mutate and develop, we have now been able to determine that the expected diameter also changes. While at the start of the pandemic in March 2020 there was still an average diameter of 50 nanometers, in mid-September 2021 the virus will already start at around 35 nanometers.
Because the various variants are present together, it is important to determine the standard deviation so that we can make this recognizable in the programming of the computer that communicates with the Nanoscan 3910.
In the QuBA we do not test whether someone is infected, but whether someone is contagious. It is therefore not the case that we can draw the conclusion “Corona” from the results of the QuBA, just as this is not the case with a PCR test. The conclusion “Corona” should at all times be determined by doctors. It is clear from the first tests in comparison with the PCR tests that there is a large correlation between a positive result of the QuBA and having Corona, after all we have a comparable result of at least 95,1%.
Because the QuBA uses the concept of “Super spreader” by making the test subjects scream, there is an abundance of sample material. Because this material can only be released after the environment in the cabin is completely clean, we are dealing with a pure measurement every time. This pure measurement is necessary because the virus or aerosol will otherwise bind itself to a particulate matter or ultrafine particulate matter. In that case, any form of certainty about determining the diameter of the virus particles would have disappeared.

The QuBA has a gatekeeper function; only those who may be contagious with a virus to avoid (Corona etc.) should be stopped. For this it is particularly important that the results of the QuBA tests have an extremely high reliability. A reliability that applies not only to a negative contagiousness but also to a positive contagiousness.

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There are two options before being tested;

1. Typical (people have Corona related complaints) and
2. A-typical (one has no complaints).

Scoring well on only 1 of these 2 possibilities makes the overall score unreliable. A test is reliable if both positive and negative cases are identified.

Because a comparison is made with a PCR test and this test is only usable after 108 hours from the moment of infection, a difference in reliability will always be visible between the QuBA and the PCR test. This is because the QuBA can already measure an infectiousness after only 36 hours from the time of infection, a difference of three days.
The speed of the QuBA benefits the reliability on the one hand, and on the other hand this will cause a significant difference in comparison with a PCR test.
Because the focus is on contagiousness and not on whether or not you are infected, two side effects virtually disappear.
The QuBA has no false positives or false negatives; someone is either contagious or not. This test disparity compared to a PCR test method can be overcome by performing a second PCR test 7 days after the first test. This was not chosen in the Dutch comparison test in connection with the far-reaching privacy legislation.
Because the results of the QuBA are compared with those of a PCR test, it is important to briefly describe the methodology of both test methods;

A PCR test uses a swab, a type of cotton swab. The swab collects material in the back of the nasal cavity and/or the throat. This is a first pitfall for the PCR test, because it is a matter of luck to take a swab/sample at the right place. In order to exclude this uncertainty, it is preferable not to take a PCR test in people who have no complaints (so-called prevalent testing). Because of this late testing method, there are many false negatives, people who think they are not contagious, but in practice they are. Because a PCR test looks for a specific protein and this is done by means of zooming in (CT values), it has been internationally agreed that a CT value of 35 and higher may no longer be seen as reliable. This is also one of the reasons that a PCR test will disappear as a reliable test method, after all, the situation in the Netherlands is that the GGD maintains a CT value of 40-45. This poses a huge issue with regard to the reliability of the QuBA test, if we have to compare it with an unreliable test such as the current PCR test
Because something needs to be said about the reliability of the QuBA test method, we use the data from the old tests, where it will mainly be about the method of measurement and the use of normal distributions.
Normal distributions are a tried and tested mathematical method, in which an average measurement value with a standard deviation can say something about the total population of measurements.
In the case of the QuBA, this standard deviation (SD), can be traced back to the diameter of the virus particle to be measured. All other possible measurable

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particles within the QuBA's measured values that fall outside the scope of interest are irrelevant and therefore have no influence on the reliability of the QuBA.

We also have to deal with the reliability of the measuring instrument in the QuBA itself (Nanoscan 3910 from TSI). According to TSI, the reliability can be read from the zero count that is higher than 99.9%.
Previous tests with the QuBA show that virus size is between 35 and 56 nanometers. This means a 100% scope spread over 21 nanometers or 3.5 SD above the average and 3.5 SD below the average of 45.5 nanometers. This means that the SD is 3 nanometers. In other words, if we limit the scan from 30 nanometers to 60 nanometers, we have at least 100% of the desired scope, so the reliability of finding the virus particle depends entirely on the reliability of the Nanoscan 3910. This means 100% times 99.9% is 99.9% reliability of the QuBA measurement.
The only remark that goes with this is that a calibration must take place every 6 months to discover whether the virus has mutated in a different diameter.
Any new parameters can then be uploaded to the already placed QuBA's.

Because the QuBA does not measure on an infection but on a high probability of infectivity of a specific virus with a known diameter, one can theoretically choose from several viruses with exactly the same diameter characteristic.
Because the Corona virus is dominant over the rest of the viruses in that specific range, the QuBA becomes a reliable diagnostic device.
Even if we were to detect another virus that comes close to the Corona virus particle, it is undesirable that the test person continues with a positive result. After all, he or she is then contagious for another virus.
Dominance of the Corona virus or any other airborne virus on which the QuBA is programmed therefore also determines 100% the result of a QuBA test.

Van Wees Innovations Europe BV

P.W. from Wees
Amsterdam, September 29, 2021

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Appendix 3 Spreadsheet test results December 2021
Zie PDF in bijlage

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Appendix 4. **Test description**

The QUBA (Quick Breath Analyzer) is a NON-INVASIVE RAPID TEST based on measuring particles which are released after shouting or singing coming from the tested person.
It's a hermetically sealed test cabin with a front door and back door (in and out). Because the air in the cabin is 99,99997% cleaned before the breath sample is taken, the detection of virus particles is very accurate.
The test procedure takes 150 seconds, in which the test person has to shout or sing for 10 seconds, to exhale as much breath as possible. After 150 seconds the test result is available.

For extensive description of the test see Appendix 5.

After the door is closed and the person is in the cabin, the air in the cabin is cleaned by sucking out the air underneath the person under the floor of the cabin and recirculated into the cabin from above. The extracted air passes through the patented Cirqulair Smogkiller unit at the back of the QuBA and reenters 100% clean. (Clean means every single polluted particle from 1 nanometer and upwards for 100%)

This cleaning procedure takes place with a capacity of 5500 m3 per hour (1,53 m3 per second). The content of the cabin is 3,2m3. Thus the cabin is cleaned completely within 3 seconds.
For safety reasons we repeat this cleaning procedure for 25 seconds to be absolutely sure that no particle is left in the cabin.
Now the second Cirqulair Smogkiller is started and the first one is shut down.
The air from an 18-liter buffer tank is passed through an air dryer to remove any possible aerosol.
After 15 seconds we do the zero measurement, concerning the entire scan on particles between 10 and 420 nanometers. This measurement is taken from the 18-liter buffer tank.
The measurement is done through a hose between the buffer tank/air dryer and the TSI SMPS device.
A picture will be taken from the zero measurement and the personal document.
Next, the client gets a signal to scream or sing for 10 seconds. During this shouting or singing tens of thousands of particles will be exhaled into the inlet of the buffer tank.
After ten seconds the buffer tank is closed and the client gets a signal to stop screaming or singing.
The operator helps the client out of the cabin and the second measurement will take place, which will also be photographed to secure the result.
If a person is contagious with a Corona virus, the scale on the SMPS device will show a normal distribution diagram around the average diameter of the virus.
The positive / negative decision is made based on the image on the SMPS device.
If there is a significant normal distribution diagram between 50 and 120 nanometers the person is called positive. If this image shows, a second run is

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done of the breath sample. If the second image is the same as the first one the conclusion is definite.

A negative result comes from the fact that no normal distribution diagram shows between 50 and 120 nanometers, on the SMPS meter.

After the person has left the cabin, the cabin is cleaned again by the first Cirqulair Smogkiller to ensure that no particles/ virus stay behind in the cabin. This is to prevent any contamination between clients. Furthermore, every two hours the cabin is cleaned with a virus terminating liquid or gel.
In the QUBA there is also an UV-C device for disinfection between the tests.

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Table Particle sizes
(source: study material Wageningen university 2020 virological lab)

	Viruses	Rickettsia	Mycoplasms	Bacteria	Fungi
Particle size	20-350 nm	200-500 nm	150-200 nm	350-5000 nm	>1000 nm

Table Resporatory Viruses (*Encyclopedia of Microbiology, Fourth Edition)*

Virus	Classification[a]	Principal syndromes	Virus detection methods
HRSV	Groups A and B	URI, bronchiolitis, croup, bronchitis, pneumonia	Culture, Ag detection, RT–PCR
HPIV	Types 1, 2, 3, 4	URI, croup, bronchiolitis, bronchitis, pneumonia	Culture, Ag detection, RT–PCR
HRV	Species A, B, and C With 100 serotypes	URI; asthma and COPD exacerbation	Culture, RT–PCR
ADV	51 serotypes	URI, PCF, bronchitis, pneumonia	Culture, Ag detection, PCR
HCoV	Types OC43, 229E, NL(NH), HKU1	URI, bronchitis, pneumonia	Culture, RT–PCR
SARS-CoV	1 type	SARS	Culture, RT–PCR
HMPV	Groups A and B	URI, bronchitis, pneumonia	Culture, RT–PCR
HBoV	2 lineages	URI, bronchiolitis, asthma exacerbation,bronchitis, pneumonia	PCR

ADV, adenovirus; Ag, antigen; HBoV, human bocavirus; HCoV, human coronavirus; HMPV, human metapneumovirus; HPIV, human parainfluenza virus; HRSV, human respiratory syncytial virus; HRV, human rhinovirus; PCF, pharyngoconjunctival fever; SARS, severe acute respiratory syndrome; SARS-CoV, Coronavirus associated with SARS; URI, upper respiratory infection; RT–PCR, reverse transcription–polymerase chain reaction, which includes both conventional and real-time methods.