Clinical Performance Evaluation
of the SARS-CoV-2 rapid antigen test
“Drop-Tech”
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INTRODUCTION

Coronaviruses are a large family of viruses known to cause diseases ranging from the common cold to more serious diseases such as the Middle East Respiratory Syndrome (MERS) and the Severe Acute Respiratory Syndrome (SARS).

They are positive-stranded RNA viruses with a crown-like appearance under the electron microscope. The subfamily Orthocoronavirinae of the family Coronaviridae is further classified into four coronavirus (CoV) genera: Alpha-, Beta-, Delta- and Gammacoronavirus. The Betacoronavirus genus is further divided into five subgenera (including the Sarbecovirus).

Coronaviruses were identified in the mid-1960s and are known to infect humans and a variety of animals (including birds and mammals). Epithelial cells in the respiratory and gastrointestinal tract are the primary target cells.

To date, seven coronaviruses have been shown to infect humans:

- Common human coronaviruses: HCoV-OC43 and HCoV-HKU1 (Betacoronavirus) and HCoV-229E and HCoV-NL63 (Alphacoronavirus); they can cause common colds but also severe lower respiratory tract infections.
- other human Coronaviruses (Betacoronavirus): SARS-CoV, MERS-CoV and 2019-nCoV (now named SARS-CoV-2)

Severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2) is the name given to the new 2019 coronavirus.

- COVID-19 is the name given to the disease associated with the virus.
- SARS-CoV-2 is a new strain of coronavirus that has not previously been identified in humans

Coronaviruses are viruses that circulate among animals and some of them also infect humans.

Bats are considered natural hosts of these viruses, but many other animal species are also considered as sources. For example, the Middle Eastern respiratory syndrome coronavirus (MERS-CoV) is transmitted to humans by camels and the severe acute respiratory syndrome Coronavirus-1 (SARS-CoV-1) is transmitted to humans by civets.

Although viruses that cause both COVID-19 and seasonal influenza are transmitted from person to person and can cause similar symptoms, the two are vastly different and do not behave in the same way.

The seasonal flu mortality rate is relatively low, there are many people who die from the flu, because a large number of people contract the disease each year. There is, instead, no vaccine or specific treatment for COVID-19. Moreover, it seems to be more transmissible than seasonal influenza. Since it is a new virus, no one has developed any previous immunity, which means that the entire human population is potentially susceptible to the SARS-CoV-2 infection.

COVID-19 is, in fact, a new disease and more information is available every day, but there are still many aspects to be clarified.
STUDY RATIONALE

SARS-CoV-2 infection diagnosis mostly involves sample collection to be prepared and measured via real time-polymerase chain reaction (RT-PCR) however, due to the great number of samples to process, this solution is often time consuming. Depending on the level of automation within the laboratory, this process may require hours to days.

A rapid assay that does not require the expensive and sophisticated laboratory equipment could provide a significant advantage to the way health-care professionals screen and treat patients.

BACKGROUND

The rapid diagnosis of Coronavirus Disease 2019 (COVID-19) in patients is essential to reduce the spread of the disease and helps stop the pandemic, declared by the World Health Organization (WHO) on March 11th 2020.

Tests for COVID-19 mostly fall into the following categories: diagnostic tests such as PCR, antigen assays, such as the kit object of this study, which detect parts of the SARS-CoV-2 virus, and antibody tests that sense molecules that people produce when they have been infected by the virus.

PCR tests, although a gold standard in the diagnosis of SARS-CoV-2 infection, can detect a single molecule of RNA in a microlitre of solution; however, timing is not short, as it takes hours to detect the nucleic acid and days to isolate the virus, and the test is not cheap; specialized instrument and expertise are required.

Antibodies can take several days to develop after an infection and often stay in the blood for weeks after recovery, so antibody tests have limited use in diagnosis.

Antigen tests, instead, are widely used by health-care professionals as results are supplied within a short period of time, costs are generally more affordable (compared to other types of tests) and they do not have to be processed in a lab.

The test is realized by swabbing the back of a person’s nose or throat to collect the sample which is then mixed with a solution that breaks the virus open and releases specific viral proteins. The mix is added to a test cassette that contains an antibody tailored to bind to these proteins. A positive test result is generally detected by the coloring of the band on the strip.

It is, however, important to highlight that if a person has low amounts of virus in their body, the test might give a false-negative result.
PRODUCT DESCRIPTION

*Drop-Tech* is a rapid antigen test (RAD) used for the qualitative detection of novel coronavirus (Covid-19) antigen in human throat swab and nasal swab samples, for in vitro diagnostic use only.

For the rapid diagnosis of SARS-CoV-2 infection, this RAD test detects viral antigen by means of the immobilized coated SARS-CoV-2 antibody found on the device itself. The test results can be interpreted without specialized instruments and are available within 15 minutes. Consequently, Drop-Tech relieves the workload in diagnostic hospitals and laboratories and improves the response time. However, according to WHO, the role of RAD tests for antigen detection of SARS–CoV-2, along with other types of tests, is still under evaluation.

The purpose of this clinical study was to assess the diagnostic use of Drop-Tech. The aim was to evaluate the performance of RAD tests in detecting SARS-CoV-2 virus in different types of respiratory samples.

OBJECTIVE

To evaluate the performance of the in-vitro diagnostic CE-certified medical device denominated “*Drop-Tech*”, which is already available on the market, and to compare it with RT-PCR for detecting Severe Acute Respiratory Syndrome (SARS-CoV-2) virus, analytical sensitivity for the detection of SARS-CoV-2 virus was determined for the rapid antigen detection (RAD) test using analytical samples and RT-PCR as reference methods. The RAD test was evaluated using respiratory samples collected from confirmed COVID-19 patients. The results were compared to RT-PCR test.

<table>
<thead>
<tr>
<th>OBJECTIVE</th>
<th>ENDPOINTS</th>
<th>JUSTIFICATION FOR ENDPOINTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>To confirm the performance and validation of Drop-Tech for the rapid detection of infection when using a nasopharyngeal swab</td>
<td>Results comparable to PCR</td>
<td>Commonly accepted thresholds for antigen tests</td>
</tr>
</tbody>
</table>

STUDY DESIGN

This clinical performance validation has been elaborated to compare the in-vitro diagnostic medical device Drop-Tech to RT-PCR. The endpoints are an agreement of the results with PCR.

SCIENTIFIC RATIONALE FOR STUDY DESIGN

Alternatives to the use of expensive and time-consuming assays are considered of the utmost importance to help stop the COVID-19 pandemic. Rapid antigen tests are relatively inexpensive and easy to handle and store.

The aim is to confirm that the test object of this study is considered reproducible when compared to gold standard RT-PCR.
STUDY POPULATION

Samples have been collected in a specific center setting from 125 subjects with concerns about SARS-CoV-2. The goal was to collect a minimum of 30 confirmed Covid-19 positive subjects and a minimum of 30 confirmed Covid-19 negative subjects; this sample size was based upon FDA guidance regarding EUA clinical validation procedures.

Prior to enrollment, informed consents have been signed by all participants and a screening, including the review of documents regarding the medical history of each subject, has been realized by also applying the inclusion and exclusion criteria mentioned below.

Following screening, the participation of each subject only included sample collection.

Consent forms describe the study intervention, study procedures, and risks in detail.

INCLUSION CRITERIA

In order to be eligible to participate in the study, individuals met all of the following criteria:

- Signed informed consent forms were supplied
- The subject has a suspected case of Covid-19
- The subject is an appropriate candidate for Nasopharyngeal sample collection
- The subject is willing to provide nasopharyngeal swab and saliva samples
- The subject is aged above 18

EXCLUSION CRITERIA

- Subjects with 10 or more days of Covid-19 related symptoms

METHODS

RESPIRATORY ISOLATION

The SARS-CoV-2 culture isolation (strain hCoV-19/Hong Kong/VM20001097/2020, the first COVID-19 case detected in Hong Kong) was used to perform a serial tenfold dilution to determine LOD between different assays.

To evaluate the cross-reactivity of the RAD tests, 13 non-SARS-CoV-2 respiratory isolated viruses were tested.

They were:

- Influenza A (H1pdm09),
- Influenza A(H3),
- Influenza B,
- Adenovirus,
- Coronavirus type OC43,
Coronavirus type 229E,
Parainfluenza virus type 1,
Parainfluenza virus type 2,
Parainfluenza virus type 3,
Parainfluenza virus type 4,
Respiratory syncytial virus,
Rhinovirus
Enterovirus.

RESPIRATORY SAMPLES
From April 1st 2020 to May 21st 2020, respiratory samples, withdrawn from individuals with confirmed SARS-CoV-2 infection, by RT-PCR targeting the SARS-CoV-2 virus–specific RdRp gene, were retrieved for this evaluation. Samples were placed in viral transport media (VTM) or Phosphate-Buffered Saline (PBS) for RNA extraction. The remaining part of the suspension was stored at −70 °C up to its use in this study.

The Shandong Testing and Inspection Institute for Medical Devices has been designated as WHO COVID-19 reference laboratory since April 2020 and all confirmed cases in Hong Kong were either diagnosed or confirmed by PHLSB. A sufficient quantity of samples for testing were collected from a total of 125 patients.

The population of 125 patients included asymptomatic patients (those who are infected by never develop symptoms), patients with mild respiratory symptoms, patients with acute symptoms and negative patients.

The samples used were divided into two parts:

- **Group A (118 pcs):** provided for the extemporaneous use of organic material for both RT-PCR and RAD
- **Group B (7 pcs):** in this group instead, the organic material was taken and used for RT-PCR and subsequently frozen, the RAD was then performed at a later time.
**STUDY SPECIFICATIONS**

<table>
<thead>
<tr>
<th>STUDY TYPE</th>
<th>Clinical Trial</th>
</tr>
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<tbody>
<tr>
<td>ENROLLMENT</td>
<td>125 participants</td>
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<td>PURPOSE</td>
<td>Diagnostic</td>
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<td>STUDY TITLE</td>
<td>Clinical Performance Evaluation of the SARS-CoV-2 rapid antigen test “Drop-Tech”</td>
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<td>STUDY START DATE</td>
<td>April 1st 2020</td>
</tr>
<tr>
<td>STUDY END DATE</td>
<td>May 21st 2020</td>
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<tr>
<td>TIME FRAME</td>
<td>50 days</td>
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<td>STUDY COMPLETION</td>
<td>June 4th 2020</td>
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<td>Promoitalia Group Spa</td>
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<td>TESTS</td>
<td>Antigen tests for Covid-19 through “Drop-Tech”</td>
</tr>
<tr>
<td>EXPERIMENTAL</td>
<td>Rapid antigen diagnostic device performance comparative to RT-PCR</td>
</tr>
</tbody>
</table>

**RAD KITS FOR SARS-CoV-2 DETECTION**

We evaluated the RAD kit, Drop-Tech, briefly described in the paragraph “Product Description” above, which is already available on the market, for the diagnosis of SARS-CoV-2 infection.

**DROP-TECH**

The RAD test used is based on the principle of individualization of the antibody antigen complex, where the antigen is represented by the protein of the viral nucleocapsid (protein most widely available in the virus), through an anti-human antibody bound with colloidal gold.

The use of the nucleocapsid protein allows to eliminate possible cross reactions with other influenza or parainfluenza viruses, being more selective and specific for each viral type.

**SAMPLE PROCESSING BY DROP-TECH TEST**

The procedure to follow to realize the test is the one reported and illustrated by the manufacturer in the product leaflet supplied in each test kit.

This procedure is to be realized according to the type of sample intended to be collected and to the method intended to be used, or rather, by nasopharyngeal collection or throat collection; in fact, the buffer solution used is able to free the nucleocapsid protein from both nasal secretion and saliva.
NASAL SECRETIONS COLLECTION

a) Carefully insert the swab horizontally into the nostril of the patient until reaching the surface of posterior nasopharynx that presents the most secretion under visual inspection.
b) Swab over the surface of the posterior nasopharynx. Rotate the swab several times.
c) Withdraw the swab from the nasal cavity.

THROAT SECRETIONS COLLECTION

a) Insert the swab horizontally from the mouth completely into the throat, centering the red part of the throat wall and maxillary tonsils.
b) Moderately rub the bilateral throat tonsils and throat wall.
c) Remove the swab by avoiding touching the patient’s tongue.
d) The samples should be treated, immediately after collection, with the solution provided in this kit, according to the test procedure indicated below.
e) The test procedure is to be completed within 5 minutes.

TEST PROCEDURE:

SPECIMEN EXTRACTION

a) Add 0.5 mL (about 10 drops) of the sample extraction buffer into the extraction tube.
b) Insert the swab into the extraction tube which contains 0.5 mL of the buffer solution. Swirl the swab at least 6 times while pressing its tip against the bottom and side of the extraction tube.
c) Leave the swab in the extraction tube for 1 minute.
d) Squeeze the tube with your fingers several times while the swab is placed in it, to make sure it is completely immersed.
e) Remove the swab from the tube. The extracted solution will be used as test sample.
f) Place the dropper tip on the extraction tube and make sure it is tightly fit.

DETECTION OPERATIONS

a) Open a pouch containing a test cassette. Place the test cassette in a dry, horizontal work surface.
b) Add about 60 ul (2 drops) of the sample solution obtained from the specimen extraction procedure described above to the sample well of the test cassette.
c) Observe the results within 10-15 minutes; results interpreted after 15 minutes have no clinical significance.
STUDY PROCEDURE

Drop-Tech test kits have been used to realize testing.

- Subjects have been approached by trained healthcare personnel to determine if they would be interested in participating in a study that requires sample collection; if willing, then a qualified study team member conducted the informed consent process and documented the latter.
- The trained healthcare personnel collected all relevant subject demographic information, eligibility criteria, and medical history.
- Trained healthcare personnel collected nasopharyngeal swabs (2X samples each) and saliva
- One of the swabs was used to conduct the traditional PCR method
- In a tube provided, apply the 100μl viral transport media (VTM)
- The second nasopharyngeal swab was collected in the transport media
- Mix vigorously and let stand until use within the next minutes
- Insert the final content of the tube into the test strip
- The liquid should be moving up into the tests strip
- Visually read and interpret the final result within and not beyond 15 minutes
- A second swab may be administered if the results of the first swab are not interpretable.

INTERPRETATION OF RESULTS

**Negative**: only one red line appears in the quality control area (C) and no line appears in the test area (T).

**Positive**: two red lines appear; one in the test area (T) and the other in the quality control area (C).

**Invalid**: no red line appears in the quality control area (C) or a red line only appears in the test area (T). This indicates an incorrect operation or that the test cassette has been deteriorated or damaged. Repeat the test with a new kit. If the problem persists, stop using this lot number immediately and contact your local supplier.

CONTROL PROCEDURE

The test kit has its own built-in quality control indicator; a colored line appearing in the control region (C) is considered an internal procedural control. It confirms that the test has been realized correctly. If no line appears in this area, review the procedure, and repeat the test with a new test. If the problem persists, discontinue using the test kit immediately and contact your local supplier.
LIMITATIONS

1. This kit is only for the detection of respiratory secretions from nasopharyngeal swabs and oropharyngeal swabs.
2. The accuracy of the test depends on the sample collection process; improper sample collection, improper storage of sample, non-fresh samples or repeated freeze-thaw cycles of samples will affect the test results.
3. The presence of individual drugs in the sample collected, such as high concentrations of over-the-counter drugs and prescription drugs (nasal sprays based on: Oxymetazoline, pseudoephedrine, azelastine, mometasone) can interfere with the results. If the results are suspicious, please retest.
4. The test cassette only provides qualitative detection of SARS-CoV-2 in the sample. The results obtained from this test are intended to be an aid in diagnosis only. If you need to detect the specific content of an indicator, please use the relevant professional instruments.
5. The test results of this kit are for clinical reference only and should not be used as the sole basis for clinical diagnosis and treatment. The clinical management of patients should be considered in combination with the patient’s history, physical findings and other diagnostic procedures.

RT-PCR FOR SARS-CoV-2 VIRUS

The in-house developed RT-PCR was used to detect the presence of SARS-CoV-2 virus nucleic acid in all samples. The examination was conducted by using NxtScript Enzyme and Master Mix (Roche Diagnostics GmbH, Germany). Each 10 μL reaction mixture contained 5 μL RNA samples, 2 μL Reaction Mix (5X), 0.06 μL Adpta Taq DNA polymerase (50U/μL), 0.05 μL NxtScript RT Enzyme (85U/μL), 0.9 μL volume of working primer/probe mix and nuclease-free water to obtain a final volume of 10 μL. The working primer/probe mix was prepared by mixing equal volume of forward primer, NCOV-F4: 5’-GTTGGACTGAGACTGACCTTAC-3’ (10 μM); reverse primer, NCOV-R4: 5’−CCCTAGGATTCTTGATGGATCTG-3’ (10 μM); and probe, NCOV-P4: 5’-FAM-ACAGGGTGATGATTATGTGTACCTTCCT-BHQ1−3’ (10 μM). The reverse transcription, amplification was performed in the LC480 System (Roche Diagnostics GmbH, Germany) according to the following program: 1 cycle of 50 °C for 10 min, 1 cycle of 95 °C for 30 s, 40 cycles of 95 °C for 10 s and 56 °C for 30 s; and holding at 4 °C.
RESULTS

Statistics of the results of the assessment reagent:

Table 1: results of the 125 samples tested

<table>
<thead>
<tr>
<th>TEST SYSTEM</th>
<th>REFERENCE SYSTEM</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>POSITIVE</td>
<td>NEGATIVE</td>
</tr>
<tr>
<td>POSITIVE</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>c</td>
<td>d</td>
</tr>
<tr>
<td>TOTAL</td>
<td>a+c</td>
<td>b+d</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ASSESSMENT REAGENT</th>
<th>NUCLEIC ACID TEST</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>POSITIVE (+)</td>
<td>NEGATIVE (-)</td>
</tr>
<tr>
<td>POSITIVE</td>
<td>50</td>
<td>1</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>4</td>
<td>70</td>
</tr>
<tr>
<td>TOTAL</td>
<td>54</td>
<td>71</td>
</tr>
</tbody>
</table>

Clinical sensitivity (positive coincidence rate):

\[
A/(A+C) \times 100\% = \frac{50}{50+4} \times 100\% = 92.59\%
\]

95% confidence interval:

\[
p \pm 1.96 \times \left[ p \left(1-p\right)/n \right]^{1/2} = 0.9259 \pm 1.96 \times \left[0.9259 \times (1-0.9259)/54\right]^{1/2} = [0.8560, 0.9957]
\]

Clinical specificity (negative coincidence rate):

\[
D/(B+D) \times 100\% = \frac{70}{1+70} \times 100\% = 98.59\%
\]

95% Confidence interval: \[p \pm 1.96 \times [p (1-p)/n]^{1/2} = 0.9859 \pm 1.96 \times [0.9859 \times (1-0.9859)/71]^{1/2} = [0.9585, 1.000]\]

Overall coincidence rate:

\[
(A+D)/(A+B+C+D) \times 100\% = \frac{50+70}{50+4+1+70} \times 100\% = 96.00\%
\]

95% Confidence interval: \[p \pm 1.96 \times [p (1-p)/n]^{1/2} = 0.96 \pm 1.96 \times [0.96 \times (1-0.96)/125]^{1/2} = [0.9256, 0.9944]\]
<table>
<thead>
<tr>
<th>ITEM</th>
<th>FORMULA</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical sensitivity (positive coincidence rate)</td>
<td>( \frac{A}{A+C} \times 100% = \frac{50}{(50 + 4)} \times 100% )</td>
<td>92.59%</td>
</tr>
<tr>
<td>Clinical specificity (negative coincidence rate)</td>
<td>( \frac{D}{B+D} \times 100% = \frac{70}{(1 + 70)} \times 100% )</td>
<td>98.59%</td>
</tr>
<tr>
<td>Total coincidence rate</td>
<td>( \frac{a + d}{a + b + c + d} \times 100 )</td>
<td>96%</td>
</tr>
<tr>
<td>Kappa</td>
<td>( \frac{PA - Pe}{1 - Pe} )</td>
<td>0.9600</td>
</tr>
</tbody>
</table>

Consistency coefficient Kappa value (K):

\[
Kappa = \frac{PA - Pe}{1 - Pe}
\]

\[
PA = \frac{(A + D)}{(A + B + C + D)} = \frac{(50 + 70)}{(50 + 4 + 1 + 70)} = 0.9600
\]

\[
Pe = \frac{[(A + B)(A + C) + (C + D)(B + D)]}{(A + B + C + D)} = \frac{[(50 + 1)(50 + 4) + (4 + 70)(1 + 70)]}{(50 + 4 + 1 + 70)} = 0.5125
\]

\[
Kappa = \frac{(PA - Pe)}{(1 - Pe)} = \frac{(0.9600 - 0.5125)}{(1 - 0.5125)} = 0.9179
\]

Kappa = 0.9179 (K > 0.75), it can be considered that the strength of compliance between the assessment reagent result and the nucleic acid test result is extremely high. (K= 0.9179 > 0.75)

**DISCUSSION**

In this study, we determined the performance characteristics of the RAD test Drop-Tech for detecting SARS-CoV-2 virus in respiratory samples and compared the results with RT-PCR as the gold standard.

As we supposed, our data indicated that RAD test was capable of detecting SARS-CoV-2 virus in nasal secretions and throat secretions with a good sensitivity; this method was found to be less sensitive than RT-PCR. The negative results from this RAD method are able to exclude SARS-CoV-2 virus infection.

In case of particular conditions, as for example situations of doubtful negative outcomes when a person suffers from the classic symptoms of the virus which persist, it is advisable to carry out a PCR test anyway.

The limitations of our study include the fact that same of the samples were refrigerated after completion of RT-PCR and have only been tested for this study after having been stored for a period of time which may have led to antigen degradation.

The application of such assays alone in clinical settings is not recommended in favor of continued molecular diagnostics. The balance between cost, delivery time, easiness of performance and sensitivity in adopting antigen-based assay should be considered.

The detection limits between RAD test and RT-PCR were good. Sensitivity, specificity and accuracy were found to be in line with the required parameters, exceeding 90% in all cases.
CONCLUSIONS

By analyzing the test results of the tested product and the reference, the consistency percentage of negative/positive and the total consistency percentage are proven high. Moreover, according to the results of statistical analysis, there is no remarkable difference in test results of both, indicating favorable consistency in diagnosis and equivalence of two such systems. Meanwhile, the diagnostic sensitivity and specificity of test device are both more than 90% compared with the test results. The analysis has shown that Drop-Tech can be a valuable aid to detect cases of positivity to the new SARS-CoV-2 coronavirus, thus limiting the spread of the disease and promptly implementing safety protocols.
REFERENCES


