COVID-19 Ag Test

For professional use

Humasis

ENG

Please read the instructions carefully before use!

[INTENDED USE]

Humasis COVID-19 Ag Test is one step in vitro diagnostic test based on an immunochromatographic assay. It is designed for qualitative detection of SARS-CoV-2 antigens in nasopharyngeal and nasal swab specimen of symptomatic patients suspected of COVID-19.

[SUMMARY AND EXPLANATION]

Coronavirus is a group of viruses that belongs to the Family Coronaviridae; a type of RNA virus of 27~32kb commonly found in birds and mammals including human. Coronavirus is divided into four genera: alpha, beta, gamma and delta. The virus causes illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV).

Coronavirus disease 2019 (COVID-19) is a new strain caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The disease originated from Wuhan city of China in December 2019.The World Health Organization (WHO) publicly named this virus 'COVID-19' and declared it a pandemic and a Public Health Emergency of International Concern. The infection is typically spread from one person to another via direct contact or respiratory droplets from cough or sneeze. Latent period from exposure to onset of symptoms is between one to fourteen days (four to seven days on average). Common symptoms and signs of infection include fever, cough, shortness of breath and breathing difficulties. In severe cases, infections can cause pneumonia, severe acute respiratory syndrome, kidney failure and even death.

Due to the wide variety of symptoms, it is difficult to differentiate COVID-19 from other existing respiratory viruses or bacteria. Diagnosing COVID-19 through isolating the virus or detecting specific genes from the collected respiratory droplet specimens is a challenge in terms of time and accessibility as it requires long hours, well-equipped laboratory and advanced technology which are often not available to many public. The test is designed to detect antigen to SARS-CoV-2, and it will help assess if an individual has COVID-19 antigen within 15 minutes in cost-effective and timely manner.

[PRINCIPLE OF THE TEST]

Humasis COVID-19 Ag Test uses monoclonal antibodies specific to COVID-19 antigens to detect COVID-19 specific antigens in human nasopharyngeal swab specimens. A nitrocellulose membrane strip in the device contains one test line and one control line. The test line is pre-coated with anti-mouse monoclonal antibody to SARS-CoV-2 Nucleocapsid and RBD for detection of SARS-CoV-2 antigens, and the control line is coated with goat anti-mouse IgG. When the extracted swab specimen is added to the sample well, it will migrate to the conjugate pad, which contains conjugated antibodies conjugated with colloidal gold directed against the SARS-CoV-2 antigen. If the sample contains SARS-CoV-2 antigens, antigen-antibody-conjugate complex will be formed. The complex will continue to migrate across the membrane until it reaches the capture zone (test line) where the complex will bind to immobilized antibodies and form visible colored band in the test line. The sample will continue to move along the membrane until it reaches the control line where excess conjugate binds and produces a second visible line. This control line indicates that the sample has migrated across the membrane as intended and the test was performed properly.

[CONTENTS]

- Test devices packaged individually in aluminum pouch (25test/box)
- Disposable test tube with extraction buffer (25ea/box)
- Filter cap (25ea/box)
- Sterilized swabs for specimen collection (25ea/box)
- Instruction for use (1ea)

[MATERIAL COMPOSITION]

- Monoclonal antibody to SARS-CoV-2 Nucleocapsid
- Monoclonal antibody specific to RBD of SARS-CoV-2 Spike Protein
- Goat anti-mouse IgG

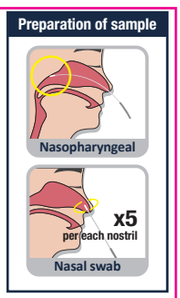
[STORAGE AND SHELF-LIFE]

- 18 months from manufacturing date at room temperature (2°C -30°C).

[TEST PROCEDURE]

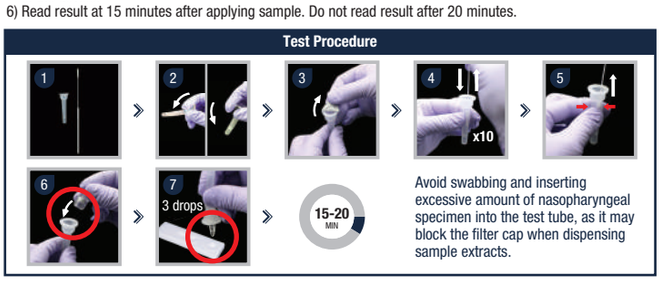
1. Specimen collection & storage:

- Nasopharyngeal swab
  1) Use the swab included in the package to collect nasopharyngeal specimen.
  2) Place the swab into one of nostrils until it reaches resistance at the posterior nasopharynx; keep it inserted for several seconds and rotate 5 times on the nasopharynx surface.
  3) Collected specimen may be stored for up to 1 hour in room temperature, and up to 4 hours in refrigerated condition (2°C-8°C). However, it is highly recommended to test the specimen immediately after collection for best results.
- Nasal swab
  1) Use the swab included in the package to collect nasal specimen.
  2) Insert the swab into left nostril up to 3/4 of an inch and firmly brush against the nasal wall in circular motion 5 times or at least 15 seconds. Proceed to do the same for right nostril.
  3) Collected specimen may be stored for up to 1 hour in room temperature, and up to 4 hours in refrigerated condition (2°C-8°C). However, it is highly recommended to test the specimen immediately after collection for best results.
- Specimen storage
  1) In case of not testing the specimen immediately after collection, the swab sample should be stored in viral transport medium (VTM) or universal transport medium (UTM).
  2) Swab sample can be stored in refrigerated condition (C) up to 12 hours, and should be frozen at -70°C after 12 hours.



2. Test method:

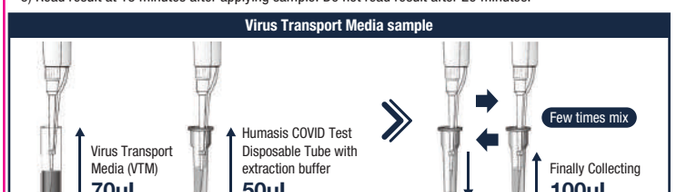
- Nasopharyngeal/ nasal swab
  1) Prepare aluminum pouch containing the test device and place it on the testing surface along with test tube and filter cap. In case the tests were refrigerated, keep them ambient for 30 minutes to let it reach the room temperature.
  2) Release the test device from aluminum pouch and place it on a level surface just prior to starting test.
  3) Shake the test tube downwards so the buffer fluid can gather on the bottom of the tube before peeling off the sealed cap. Insert the tip of the swab into the test tube and shake the tip up and down inside the tube more than 10 times to make sufficient sample extraction.
  4) Remove the swab while squeezing the test tube.
  5) Equip the filter cap on the test tube and dispense 3 drops of sample extracts (90~100uL) into the sample well of the device.
  6) Read result at 15 minutes after applying sample. Do not read result after 20 minutes.



- Nasopharyngeal swab in VTM

(Prepare empty tube and micropipette prior to testing as they are required but not provided with test components)

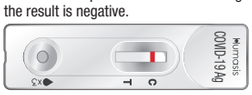
1) Take out the sample from refrigerator/ freezer and keep them ambient for 30 minutes to let it reach the room temperature.
2) Peel off the sealed cap of the test tube, and take 50uL of the extraction buffer into the empty tube using micropipette.
3) Mix the specimen in VTM well using micropipette, and take out 70uL of the specimen and transfer into the tube prepared at step 2) and mix well.
4) Prepare aluminum pouch containing the test device and place it on the testing surface along with test tube and filter cap. In case the tests were refrigerated, keep them ambient for 30 minutes to let it reach the room temperature.
5) Release the test device from aluminum pouch and place it on a level surface just prior to starting test.
6) Dispense 100uL of the prepared sample into the sample well of the device using micropipette.
6) Read result at 15 minutes after applying sample. Do not read result after 20 minutes.



[INTERPRETATION OF RESULT]

Negative

If no colored line appears in the test line (T) and a colored line is present on the control region (C), then the result is negative.

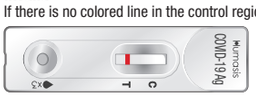


Positive

If colored line is visible in the test line (T) and control line (C), the result is positive.

Invalid

If there is no colored line in the control region (C), the result is invalid.



[PERFORMANCE CHARACTERISTICS]

- **Limit of detection (LoD)**

The limit of detection (LoD) of Humasis COVID-19 Ag Test is $5x10^{0.8}TCID_{50}$/mL.

- **Precision**

4 individual studies were performed: repeatability (within-laboratory precision), between-operator precision, between-lot precision and between-place precision of the Humasis COVID-19 Ag Test. The test results confirmed that the Humasis COVID-19 Ag Test shows consistent performance within laboratory, between operators, between lots and between places, and all the results showed 100% agreement with the expected results.

- **Reactivity / Inclusivity**

Reactivity to the following recombinant antigens in which each important amino acid of Spike RBD was mutated were confirmed: SARS-CoV-2 Spike RBD(S477N), SARS-CoV-2 Spike RBD(N501Y), SARS-CoV-2 Spike RBD(L452R), SARS-CoV-2 Spike RBD(E484K) and SARS-CoV-2 Spike RBD (K417N, E484K, N501Y) showed reactivity to 100pg/mL.

- **Cross-reactivity**

Below potential cross-reactive substances did not affect performance of the Humasis COVID-19 Ag Test.

Virus (≥10^5 PFU/mL).							
1	Coronavirus OC43	6	Human adenovirus 3	11	Parainfluenza 1	16	Metapneumovirus
2	Coronavirus 229E	7	Human adenovirus 5	12	Parainfluenza 2	17	Human Enterovirus
3	Coronavirus NL63	8	Human adenovirus 7	13	Parainfluenza 3	18	Influenza A H1N1
4	MERS-coronavirus	9	Respiratory syncytial virus A	14	Parainfluenza 4a	19	Influenza A H3N2
5	Human adenovirus 1	10	Respiratory syncytial virus B	15	Rhinovirus 1	20	Influenza B
Bacteria (≥10^6 CFU/mL)							
21	*Mycoplasma pneumonia Ag*	24	*Streptococcus pneumoniae*	27	*Candida albicans*	30	*Staphylococcus aureus*
22	*Streptococcus pyogenes*	25	*Legionella pneumophila*	28	*Chlamydia pnuemoniae*	31	*Enterococcus casseliflavus*
23	*Bordetella pertussis*	26	*Haemophilus influenzae*	29	*Staphylococcus epidermidis*	-	
Others (100%)							
32	Pooled human nasal wash – to represent diverse microbial flora in the human respiratory tract						

- **Interference**

Below potential interfering substances did not affect performance of the Humasis COVID-19 Ag Test.

No.	Interfering substances	Concentration	No.	Interfering substances	Concentration
1	Whole blood	4%	24	K3-EDTA	20 mg/mL
2	Mucin	0.5%	25	Diphenhydramine hydrochloride	5 mg/mL
3	Chloraseptic	1.5 mg/mL	26	Acetaminophen	199 µmol/L
4	NeilMed NasoGel	5% v/v	27	Acetylsalicylic acid	3.62 mmol/L
5	CVS Nasal drops	15% v/v	28	Ibuprofen	2.425 mmol/L
6	Afrin (Oxymetazoline)	15% v/v	29	Olopatadine hydrochloride	5 mg/mL
7	Sodium cromoglycate (CVS nasal spray, Cromolyn)	15% v/v	30	Hanmi Ko-and-Cool Nasal Spray (Chlorpheniramine Maleate 250 mg/ 100 mL, Xylometazoline Hydrochloride 0.1 g/100 mL)	10%(v/v)
8	Zicam	15% v/v			
9	Homeopathic (Alkalol)	1:10 dilution	31	Samchundang Narista-S Nasal Spray (Chlorpheniramine Maleate 2.5 mg/mL, Dipotassium Glycyrrhizinate 3 mg/mL, Naphazoline Hydrochloride 0.5 mg/mL)	10%(v/v)
10	Sore throat Phenol Spray	15% v/v			
11	Tobramycin	5 µg/mL			
12	Mupirocin	10 mg/mL			
13	Fluticasone Propionate	5% v/v	32	Sodium chloride	20 mg/mL
14	Tamiflu (Oseltamivir Phosphate)	5 mg/mL	33	Zanamivir	5 mg/mL
15	Albumin, human	3000 mg/dL	34	Oseltamivir	10 mg/mL
16	Bilirubin	500 µmol/L	35	Artemether-lumefantrine	50 µmol/L
17	Hemoglobin	500 mg/dL	36	Doxycycline hyclate	70 µmol/L
18	Cholesterol	20 µmol/L	37	Quinine	150 µmol/L
19	Triglycerid	1000 mg/dL	38	Lamivudine	1 mg/mL
20	Biotin	0.75 mg/mL	39	Erythromycin	81.6 µmol/L
21	Sodium citrate	25 mg/mL	40	Ciprofloxacin	30.2 µmol/L
22	Heparin	100 U/mL	41	Rheumatoid factor positive plasma	10%(v/v)
23	EDTA	5 µmol/L	-		

[CLINICAL EVALUATION]

- **Clinical evaluation**

A total of 3 studies were conducted for clinical evaluation of the Humasis COVID-19 Ag Test: retrospective study using nasopharyngeal swab sample, prospective study using nasopharyngeal sample and prospective study using nasal swab sample. The following table summarizes the clinical performance of the Humasis COVID-19 Ag Test compared against the FDA EUA approved RT-PCR assay at multiple sites.

Clinical evaluation summary		Retrospective study using nasopharyngeal swab	Prospective study using nasopharyngeal swab	Prospective study using nasal swab
Total sample (n)		510	530	77
RT-PCR positive (n)		231	123	43
RT-PCR negative (n)		279	407	34
Positive agreement % (n) (95% CI)		91.3% (211/231) (87.0-94.3%)	93.5% (115/123) (87.7-96.7%)	93.0% (40/43) (81.4-97.6%)
Positive agreement % by ct value	Ct ≤ 24 (95% CI)	100% (132/132) (97.2-100%)	100% (63/63) (92.3%-100.0%)	-
	Ct ≤ 27 (95% CI)	98.8% (161/163) (95.6-99.7%)	98.8% (82/83) (93.5-99.8%)	-
	Ct ≤ 30 (95% CI)	98.4% (179/182) (95.3-99.4%)	97.0% (96/99) (91.5-99.0%)	-
	Ct > 30 (95% CI)	65.3% (32/49) (51.3-77.1%)	79.2% (19/24) (59.5-90.8%)	-
Positive agreement % by days from symptom onset	0-4 days (95% CI)	92.6% (112/121) (86.5-96.0%)	96.3% (78/81) (89.7-98.7%)	-
	5-7 days (95% CI)	85.0% (34/40) (70.9-92.9%)	88.1% (37/42) (75.0-94.8%)	-
Negative agreement % (n) (95% CI)		100% (279/279) (98.6-100%)	99.5% (405/407) (98.2-99.9%)	100% (34/34) (89.8-100%)

* Days from symptom onset data were collected only in India, Kazakhstan and Algeria cohorts for retrospective study using nasopharyngeal swab specimen.

** Ct value and days from symptom onset data is being collated for prospective study using nasal swab specimen.

[PRECAUTIONS AND LIMITATIONS]

- For in vitro diagnostic use only
- Do not use the test device beyond the expiration date.
- Keep sealed until usage, and once opened use immediately.
- Do not use the test device if the pouch is damaged or the device is seriously broken.
- Do not re-use the device.
- Handle all specimens safely as potentially infectious.
- This test is intended for initial screening of coronavirus infection by detecting COVID-19 antigen, but should not be used as a sole criterion for the determination of SARS-CoV-2 infection. Other methods and clinical information (signs and symptoms) should be used and considered for diagnosis

[REFERENCES]

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[3] Kang CK, Song KH, Choe PG, et al. Clinical and Epidemiologic Characteristics of Spreaders of Middle East Respiratory Syndrome Coronavirus during the 2015 Outbreak in Korea. J Korean Med Sci 2017; 32:744-9.
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REF ACOVA-7025

IVD : For *in vitro* diagnostic use
LOT : Lot number
REF : Catalogue number
: Consult instructions for use
: Store at 2~30°C
: Do not reuse
EC REP : Authorized Representative
: Manufactured by
EXP : Use by / Expiry date
CE : This product fulfills the requirements for Directive 98/79/EC on *in vitro* diagnostic medical devices

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