Amazingbio COVID-19 Antigen Sealing Tube Test Strip (Colloidal Gold)
Analitical Performance Evaluation Report

- **Study Objective**

  To evaluate the analytical performance of the Amazingbio COVID-19 Antigen Sealing Tube Test Strip (Colloidal Gold).

- **Primary Investigator**

Matthew Chan, BS; Hua Li, PhD, DABCC
PacGenomics Clinical Genetics Laboratory
28118 Agoura Road, Ste200
Agoura Hills, CA, 91301 USA
Phone: (818) 597-1938

PacGenomics Clinical Genetics Laboratory is a CLIA-certified/CAP-accredited clinical laboratory.
CLIA Number: 05D2047289
CAP Number: 8658013

- **Product to be Evaluated**

  Amazingbio COVID-19 Antigen Sealing Tube Test Strip (Colloidal Gold)
  Manufacturer: Amazing Biotech (Shanghai) Co., LTD.
  Lot: G21041701
  Manufacturing date: 4/17/2021
  Exp date: 10/16/2022

- **Product Intended Use**

  Amazing COVID-19 antigen sealing tube test strip is a lateral flow immunochromatographic assay for in vitro qualitative detection of COVID-19 nucleocapsid protein antigen in human nasopharyngeal, nasal secretion, saliva or/and serum specimens directly collected from individuals suspected of COVID-19 by their healthcare provider within the first seven days of symptom onset. Results from the Amazing COVID-19 antigen sealing tube test strip should not be used as the sole basis for diagnosis. The kit is as an assistance for diagnostic and epidemiological investigation. The kit is intended for use by medical professionals or trained operators who are proficient in performing tests and trained clinical laboratory personnel or individuals trained in point of care settings.

- **Test Principle**

  Amazing COVID-19 antigen sealing tube test strip is based on sandwich lateral flow immunochromatographic assay highly sensitively detecting COVID-19 nucleocapsid protein in human nasopharyngeal, nasal secretion, saliva or/and serum specimens, having an invisible T (test) zone and C (control) zone. When sample is applied into the test tube, the liquid will laterally flow on the surface of the test strip. If there is enough COVID-19 antigen in the sample, a visible T band will appear. The C band should always appear after a sample is applied, indicating a valid result. By this means, the device can accurately indicate the presence of COVID-19 antigen in the sample.

- **Components of the Diagnostic Kit**

  The test kit consists:

  1. Sealing tube test strip with sealed diluent, 20 kits in a pouch per box
  2. Sample swab, 20 per box
  3. One instruction for use, per box

- **Storage conditions and Shelf life**

  The kit can be stored at room temperature (4-30°C). It is stable through the expiration date (18 months) marked on the foil pouch. Please seal the pouch carefully if batch tube still left inside and should use it up in one week once the pouch is opened.

  DO NOT FREEZE. Do not store the test kit in direct sunlight.

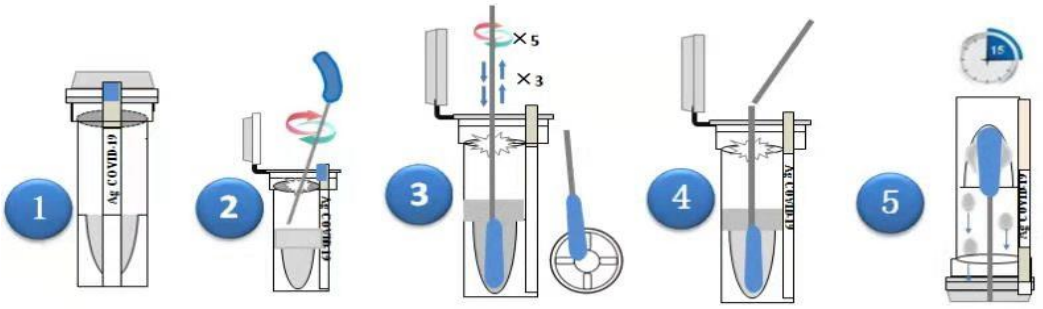
- **Specimen Requirements**

  Nasopharyngeal (NP) swab collection: Tilt patient's head back 70 degrees. Gently and slowly insert swab through the nostril parallel to the palate (not upwards) until resistance is encountered or the distance is equivalent to that from the ear to the nostril of the patient, indicating contact with the nasopharynx. Gently rub and roll the swab. Leave swab in place for several seconds to absorb secretions. Slowly remove swab while rotating it. Specimens can be collected from both nostrils using the same swab. Place swab, tip first, into the transport collection tube provided. Label the tube and place inside the small biohazard bag and seal the top of the bag. Place the small biohazard bag with the collected specimen into a larger second biohazard collection bag and seal the top of the bag.

  Nasal swab collection: Insert the entire collection tip of the swab provided (usually ½ to ¾ of an inch, or 1 to 1.5 cm) inside the nostril. Firmly sample the nasal wall by rotating the swab in a circular path against the nasal wall at least 4 times. Take approximately 15 seconds to collect the specimen. Be sure to collect any nasal drainage that may be present on the swab. Repeat in the other nostril using the same swab. Place swab, tip first, into the transport tube provided. Label the tube and place inside the small biohazard bag and seal the top of the bag. Place the

small biohazard bag with the collected specimen into a larger second biohazard collection bag and seal the top of the bag.

- **Test Procedure**

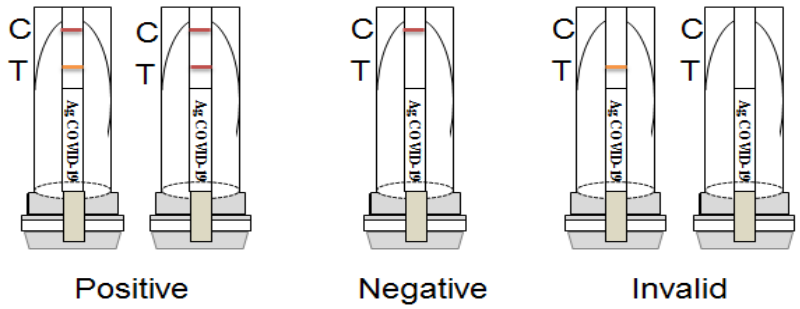


1. Take out the kit and equilibrate to room temperature (20~25°C).Open the foil pouch, take needed sealing tube test strips for test and seal the pouch carefully. Please use it up in one week once the pouch is opened. Write the patient's ID on the tube if required.
2. Carefully observe the top of the strip from outside, if the sensing paper changes to pink or white, please discard; if it remains blue go to next step 1
3. Prepare for taking sample. To remove any excess mucus, gently blow your nose into a tissue and discard it into a closed bin.
4. Take out the swab from the bag and use the end of the swab pole to break the aluminum foil. Make sure the hole is big enough. 2
5. Collect the sample. Carefully insert the swab tip around 2.5cm into one nostril until you feel a slight resistance. Roll the swab firmly around the inside of the nostril, making 10 complete circles. Repeat with the other nostril using the same swab.
6. Insert the sampled swab into the tube where there is a centre hole surrounded by four inner walls. Rotate the tip of the swab against the inner walls 5 times and rub the swab tip in an up and down motion against the inner walls 3 times 3
7. Break the TouchsoftTM swab and leave the tip in the tube 4
8. Turn the tube upside down and gently knock the cap on the table once, so that all the liquid reaches the tube cap and fully contact with the strip. The buffer will migrate along the strip and the liquid will rise up the white membrane 5

9. Interpret the result after 15 minutes referring to the Reference card. (The strip in this creative sealing tube can avoid false signal caused by back migration of strip in the cassette).

- **Interpretation of results**

  The detection results of the kit are interpreted according to the following.



Negative: Only clear C band appears, which means no COVID-19 antigen was detected

Positive: The presence of both C band and T band, no matter T band is clear or vague, which means that COVID-19 antigen was detected.

Invalid: No colored band appears in C zone, no matter whether T band appears. It is necessary to retest with a new tube test strip.

- **Limitations**

1. Negative result may occur if the level of antigen in sample is below the test LoD.

2. Negative result, from patient with symptom onset beyond seven days, should be treated as presumptive and confirmation with a molecular assay, if necessary, patient management may be performed.

3. Positive test results do not differentiate between SARS-CoV and COVID-19\, nor do not rule out co-infections with other pathogens.

4. False negative may occur if specimen is improperly collected, transported or handled, if inadequate extraction buffer is used (e.g., <12 drops)., or if specimen swab is not twirled within the test tube.

5. If the differenetation of specific SARS virus and strain is needed, additional testing, in consultation with state local public health departments, is require.

- **Limit of Detection (LoD) - Analytical Sensitivity:**

The Limit of Detection (LoD) of the Amazingbio COVID-19 Antigen Sealing Tube Test Strip (Colloidal Gold) was determined using limiting dilutions of gamma irradiation inactivated SARS-CoV-2 (BEI Resources, Manassas, VA). The BEI Resources material is a preparation of SARS-Related Coronavirus 2 (SARS-CoV02), isolated USA-WA1/2020 (Catalog No. NR-52287, Lot: 70039068) that has been inactivated by gamma irradiation. The substance was supplied frozen at a concentration of 2.8×10^6 $TCID_{50}$/mL.

The strain was spiked into the pooled swab matrix obtained from multiple healthy volunteers (negative COVID-19 confirmed by RT-PCR method) eluted in sample extraction buffer and prepare positive samples. SARS-CoV-2 was diluted according to the dilution ratio on the table below; serial dilutions of the characterized SARS-CoV-2 were then tested in three (3) replicates. The lowest concentration at which all 3 replicates were positive was treated as the tentative LoD for each test. The LoD of each test was then confirmed by testing 20 replicates with concentrations at the tentative limit of detection. The final LoD of each test was determined to be the lowest concentration resulting in positive detection of 20 out of 20 replicates.

The confirmed LoD for Amazingbio COVID-19 Antigen Sealing Tube Test Strip (Colloidal Gold) using a reference control gamma irradiation inactivated SARS-CoV-2 was 312 $TCID_{50}$/mL. The tables below summarizes the data from the LoD study:

Table 1 Estimation of LoD

Spiking Concentration ($TCID_{50}$/mL)	**Tests (Triplicates)**		
	1	**2**	**3**
10,000	+	+	+
5000	+	+	+
2500	+	+	+
1250	+	+	+
625	+	+	+
312	+	+	+
156	+	+	+
78	-	-	-

Table 2 Verification of LoD at 312 $TCID_{50}$/mL

Tests	Concentration (312 $TCID_{50}$/mL)	Concentration (156 $TCID_{50}$/mL)
1	+	+
2	+	+
3	+	+
4	+	+
5	+	+
6	+	-
7	+	+
8	+	+
9	+	+
10	+	+
11	+	-
12	+	+
13	+	+
14	+	+
15	+	+
16	+	-
17	+	+
18	+	+
19	+	+
20	+	+

- **Cross reactivity (Analytical Specificity) Study:**

  The potential cross-reactivity of the Amazingbio COVID-19 antigen sealing tube test strip (Colloidal Gold) was evaluated by testing twenty-seven (27) commensal and pathogenic microorganisms (Wet testing: 16 viruses, 9 bacteria, 1 yeast, and 1 pooled human nasal wash) that may be present in the nasal cavity. Each organism, virus, bacteria, and yeast were tested in triplicate (3) in the absence or presence of 3x LoD of the heat-inactivated SARS-CoV-2 virus (NR-52287). No cross reactivity or interference was seen with the following microorganisms when tested at the concentration presented in the table below.

Table 3 Amazingbio COVID-19 Antigen Sealing Tube Test Strip (Colloidal Gold) Cross Reactivity

Virus/Bacteria/Parasite name	Strain	Wet-testing Concentration/ *In silico* testing	Cross Reactivity Results (Number of Positive/Total)
Human coronavirus	229E	1.6 x $10^{5.0}$ $TCID_{50}$/mL	No Cross-Reactivity (0/3)
Human coronavirus	OC43	1.6 x $10^{5.0}$ $TCID_{50}$/mL	No Cross-Reactivity (0/3)
Human coronavirus	NL63	1.6 x $10^{3.0}$ $TCID_{50}$/mL	No Cross-Reactivity (0/3)
MERS-coronavirus	EMC/2012	1.6 x $10^{5.0}$ $TCID_{50}$/mL	No Cross-Reactivity (0/3)
Adenovirus	Serotype 5	1.6 x $10^{5.0}$ $TCID_{50}$/mL	No Cross-Reactivity (0/3)
Human metapneumovirus (hMPV)	TN/91-320	1.6 x $10^{5.0}$ $TCID_{50}$/mL	No Cross-Reactivity (0/3)
Human parainfluenza virus 1	HPIV1/FRA/292211 06/2009	8.9 x $10^{5.0}$ $TCID_{50}$/mL	No Cross-Reactivity (0/3)
Human parainfluenza virus 2	Greer	1.0 x $10^{5.0}$ $TCID_{50}$/mL	No Cross-Reactivity (0/3)
Human parainfluenza virus 3	NIH 47885	1.6 x $10^{5.0}$ $TCID_{50}$/mL	No Cross-Reactivity (0/3)
Human parainfluenza virus 4a	M-25	1.6 x $10^{3.0}$ $TCID_{50}$/mL	No Cross-Reactivity (0/3)
Human parainfluenza virus 4b	19503	5.0 x $10^{5.0}$ $TCID_{50}$/mL	No Cross-Reactivity (0/3)
Influenza A	A/California/07/2009	5.2 x $10^{5.0}$ $TCID_{50}$/mL	No Cross-Reactivity (0/3)
Influenza B	B/Hong Kong/330/2001 (Victoria Lineage)	1.8 x $10^{5.0}$ $TCID_{50}$/mL	No Cross-Reactivity (0/3)
Enterovirus	71/Tainan/4643/98	1.6 x $10^{5.0}$ $TCID_{50}$/mL	No Cross-Reactivity (0/3)
Respiratory Syncytial Virus A	1998/12-21	2.8 x $10^{5.0}$ $TCID_{50}$/mL	No Cross-Reactivity (0/3)
Rhinovirus	16	>5.0 x $10^{3.0}$ $TCID_{50}$/mL	No Cross-Reactivity (0/3)
Haemophilus influenzae	Type B CK	>$10^{4.0}$ cfu/vial*	No Cross-Reactivity (0/3)
Streptococcus pneumoniae	Z022	3.6 x $10^{6.0}$ cfu/mL	No Cross-Reactivity (0/3)
Streptococcus pyogenes	Z018/M58	3.9 x $10^{6.0}$ org/mL	No Cross-Reactivity (0/3)
Pooled human nasal wash	N/A	N/A	No Cross-Reactivity (0/3)
Bordetella pertussis	18323 [NCTC 10739]	4.8 x $10^{6.0}$ cells/mL	No Cross-Reactivity (0/3)

Virus/Bacteria/Parasite name	Strain	Wet-testing Concentration/ *In silico* testing	Cross Reactivity Results (Number of Positive/Total)
Mycoplasma pneumoniae	M129	2.7 x $10^{6.0}$ cfu/mL	No Cross-Reactivity (0/3)
Chlamydia pneumoniae	TW-183	9.1 x $10^{6.0}$ IFU/mL	No Cross-Reactivity (0/3)
Legionella pnuemophila	Philadelphia	1.9 x $10^{6.0}$ cfu/mL	No Cross-Reactivity (0/3)
Staphylococcus aureus	MRSA	6.5 x $10^{5.0}$ cfu/mL	No Cross-Reactivity (0/3)
Staphylococcus epidermidis	MSSE;HER 1292	7.7 x $10^{6.0}$ cfu/mL	No Cross-Reactivity (0/3)
Candida albicans	Y537	5.0 x $10^{6.0}$ cfu/mL	No Cross-Reactivity (0/3)

* No quantitation data provided. The stock concentration on the Certificate of Analysis (CoA) was >10^4 cfu/vial.
** IFU/mL is Infectious Units per milliliter.

Table 4 Amazingbio COVID-19 Antigen Sealing Tube Test Strip (Colloidal Gold) organisms interference

Virus/Bacteria/Parasite name	Strain	Wet-testing Concentration	Interference Results (Number of Positive/Total)
Human coronavirus	229E	1.6 x $10^{5.0}$ $TCID_{50}$/mL	No Interference (3/3)
Human coronavirus	OC43	1.6 x $10^{5.0}$ $TCID_{50}$/mL	No Interference (3/3)
Human coronavirus	NL63	1.6 x $10^{3.0}$ $TCID_{50}$/mL	No Interference (3/3)
MERS-coronavirus	EMC/2012	1.6 x $10^{5.0}$ $TCID_{50}$/mL	No Interference (3/3)
Adenovirus	Serotype 5	1.6 x $10^{5.0}$ $TCID_{50}$/mL	No Interference (3/3)
Human metapneumovirus (hMPV)	TN/91-320	1.6 x $10^{5.0}$ $TCID_{50}$/mL	No Interference (3/3)
Human parainfluenza virus 1	HPIV1/FRA/292211 06/2009	8.9 x $10^{5.0}$ $TCID_{50}$/mL	No Interference (3/3)
Human parainfluenza virus 2	Greer	1.0 x $10^{5.0}$ $TCID_{50}$/mL	No Interference (3/3)
Human parainfluenza virus 3	NIH 47885	1.6 x $10^{5.0}$ $TCID_{50}$/mL	No Interference (3/3)
Human parainfluenza virus 4a	M-25	1.6 x $10^{3.0}$ $TCID_{50}$/mL	No Interference (3/3)
Human parainfluenza virus 4b	19503	5.0 x $10^{5.0}$ $TCID_{50}$/mL	No Interference (3/3)
Influenza A	A/California/07/2009	5.2 x $10^{5.0}$ $TCID_{50}$/mL	No Interference (3/3)

Virus/Bacteria/Parasite name	Strain	Wet-testing Concentration	Interference Results (Number of Positive/Total)
Influenza B	B/Hong Kong/330/2001 (Victoria Lineage)	1.8 x $10^{5.0}$ $TCID_{50}$/mL	No Interference (3/3)
Enterovirus	71/Tainan/4643/98	1.6 x $10^{5.0}$ $TCID_{50}$/mL	No Interference (3/3)
Respiratory Syncytial Virus A	1998/12-21	2.8 x $10^{5.0}$ $TCID_{50}$/mL	No Interference (3/3)
Rhinovirus	16	>5.0 x $10^{3.0}$ $TCID_{50}$/mL	No Interference (3/3)
Haemophilus influenzae	Type B CK	>$10^{4.0}$ cfu/vial*	No Interference (3/3)
Streptococcus pneumoniae	Z022	3.6 x $10^{6.0}$ cfu/mL	No Interference (3/3)
Streptococcus pyogenes	Z018/M58	3.9 x $10^{6.0}$ org/mL	No Interference (3/3)
Pooled human nasal wash	N/A	N/A	No Interference (3/3)
Bordetella pertussis	18323 [NCTC 10739]	4.8 x $10^{6.0}$ cells/mL	No Interference (3/3)
Mycoplasma pneumoniae	M129	2.7 x $10^{6.0}$ cfu/mL	No Interference (3/3)
Chlamydia pneumoniae	TW-183	9.1 x $10^{6.0}$ IFU/mL	No Interference (3/3)
Legionella pnuemophila	Philadelphia	1.9 x $10^{6.0}$ cfu/mL	No Interference (3/3)
Staphylococcus aureus	MRSA	6.5 x $10^{5.0}$ cfu/mL	No Interference (3/3)
Staphylococcus epidermidis	MSSE;HER 1292	7.7 x $10^{6.0}$ cfu/mL	No Interference (3/3)
Candida albicans	Y537	5.0 x $10^{6.0}$ cfu/mL	No Interference (3/3)

- **Endogenous Interference Substances Studies:**

  Endogenous interference substances studies of the Amazingbio COVID-19 Antigen Sealing Tube Test Strip (Colloidal Gold) were evaluated by testing the following substances listed in the table below. Each substance was tested in triplicate (3) with 3x LoD, 2250 $TCID_{50}$/mL, inactivated SARS-CoV-2 virus (NR-52287). All samples tested produced expected results, demonstrating that the Amazingbio COVID-19 Antigen Sealing Tube Test Strip (Colloidal Gold) performance was not affected by any of the 14 potentially interfering substances listed in the table 5 below.

Table 5 Endogenous interference study of Amazingbio COVID-19 Antigen Sealing Tube Test Strip (Colloidal Gold)

Potential Interfering Substances	Active Ingredient	Test concentration	Results (Number of Positive/Total)
Whole Blood	Blood (human)	4 % V/V	No Interference (3/3)
Mucin	Mucin protein, Type I-S	0.5 % W/V	No Interference (3/3)
Chloraseptic	Benzocaine, Menthol	0.15 % W/V (1.5mg/ml)	No Interference (3/3)
Naso Gel (NeiMed)	Saline	5.0 % V/V	No Interference (3/3)
Nasal Spray	Phenylephrine	15.0 % V/V	No Interference (3/3)
Afrin	Oxymetazoline	15.0 % V/V	No Interference (3/3)
Zicam	Oxymetazoline, Hydrochloride	5.0 % V/V	No Interference (3/3)
Nasal Spray (Cromolyn)	Cromolyn sodium	15.0 % V/V	No Interference (3/3)
Alkalol	Galphimia glauca, Luffa operculata, Sabadilla	1:10 dilution	No Interference (3/3)
Sore Throat Phenol Spray	Phenol	15.0 % V/V	No Interference (3/3)
Tobramycin	Tobramycin	0.0004% W/V (4ug/ml)	No Interference (3/3)
Mupirocin	Mupirocin	1.0 % W/V (10mg/ml)	No Interference (3/3)
Fluticasone Propionate	Fluticasone propionate (glucocorticoid)	5.0 % V/V	No Interference (3/3)
Tamiflu	Oseltamivir	0.5 % W/V (5 mg/mL)	No Interference (3/3)

- **High-dose Hook Effect:**

The high-dose hook effect of the Amazingbio COVID-19 Antigen Sealing Tube Test Strip (Colloidal Gold) was evaluated by testing the following serial dilutions of the characterized SARS-CoV-2 listed in the table. No high dose hook effect was observed when tested with up to a concentration of $1.5 \times 10^{5.0}$ or $1.0 \times 10^{5.0}$ $TCID_{50}$/mL of the inactivated SARS-CoV-2 virus (NR-52287) to the Hercin 2019-nCoV Antigen Test Kit.

Table 6 High-dose hook effect of Amazingbio COVID-19 Antigen Sealing Tube Test Strip (Colloidal Gold)

SARS-CoV-2 Concentration ($TCID_{50}$/mL)	High-dose Hook Effect Test Results (Number of Positive/Total)
$1.5\times10^{5.0}$	Not Identified (3/3)
$1.0\times10^{5.0}$	Not Identified (3/3)

This report was reviewed and approved by PacGenomics.

Co-Lab Director: Hua Li, PhD, DABCC Date: May 29, 2021

Date: May 29, 2021

About PacGenomics Clinical Genetics Laboratory:

PacGenomics, a fully accredited clinical genetics laboratory, we use our ingenuity to create assays that generate the highest resolution and clinical sensitivity. Our innovative scientists are continually working to improve the field of reproductive genetic testing by providing high-quality NGS based testing with excellent client care and support to match. PacGenomics offers Viral PCR Testing and Antibody Testing for COVID-19.

Address: PacGenomics, 28222 Agoura Road, Suite 200/201,
Agoura Hills, CA 91301, USA

Office Phone: +1 818-597-1938
Office Fax: +1 818-597-1939
http://www.pacgenomics.com