Anbio Rapid COVID-19 Antigen Test (Colloidal Gold) / Saliva

Clinical Trial Analysis Report

Report No:	Anbio-Saliva-20210417
Project Name:	Anbio Rapid COVID-19 Antigen Test (Colloidal Gold) / Saliva Clinical Trial Analysis
Type of Clinical Trial:	Clinical Trial Analysis Validation
Clinical Trial Institution:	PacGenomics Clinical Genetics laboratory
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Collection Site:	Hollywood Hills Women's Medical Group
Address:	9201 W Sunset Blvd, Suite 401, West Hollywood, CA 90069
Principal Investigator:	Dr. Karen Lapesarde
Tel:	+1 818-597-1938
Email:	labsite@hhwmg.com
Manufacturer:	Anbio (Xiamen) Biotechnology Co., Ltd.
Address:	No.2016, Wengjiao West Road, Xinyang Street, Haicang District, Xiamen, Fujian, China
Tel	+86 592 6312 399
Email:	info@anbio.com
Start Date of Clinical Trial:	03/25/2021
Completion Date of Clinical Trial:	04/17/2021

Abstract

Study Summary

A study was performed at **PacGenomics Clinical Genetics laboratory** to evaluate the test kits performance from 3/25/2021-4/17/2021. Samples obtained from travelers including European travelers were used for the study. All samples are blinded and randomly mixed that the operator does not have prior bias about the result. A total of 420 samples obtained from travelers including 205 European travelers were tested during the study. The samples were tested with the detection reagent and the reference reagent respectively, and the detection results of the two products were compared. Perform statistical analysis on the test results calculate the percentage of diagnostic specificity and sensitivity to the total coincidence rate. According to the statistical analysis results, the applicability and accuracy of the Test Kit are judged, so as to determine whether the test results of the Test Kit are consistent with the test results of the control Kit.

The results of Anbio Rapid COVID-19 Antigen Test (Colloidal Gold) / Saliva showed a good agreement with PCR and clinical results. The clinical Sensitivity of Anbio Rapid COVID-19 Antigen Test (Colloidal Gold) / Saliva is 99.06%, clinical Specificity is 100%. In conclusion, the **Anbio Rapid COVID-19 Antigen Test (Colloidal Gold) / Saliva** has good clinical performance, and meets the clinical requirement.

Dr. Li is a study Coordinator / Supervisor to supervise the entire study process and witness the whole process was done correctly including sample collection, sample extraction, adding and results reading.

CONTENS

I. Introduction

The COVID-19 antigen rapid test is a colloidal gold immunochromatography test for the qualitative detection of nucleocapsid antigens from SARS-CoV-2 in human saliva. It is suitable for use by people who are suspected of having a COVID-19 disease for users over 18 years of age. Children and adolescents under the age of 18 should only take the test under adult supervision.

The new coronaviruses belong to the genus β-corona viruses. The disease they cause, COVID-19, is an acute infectious disease of the respiratory tract. Currently, people infected with the novel coronavirus are the main source of infection, but asymptomatically infected people can also be infectious. The incubation period is 1 to 14 days, usually 3 to 7 days. The main symptoms are fever, tiredness and a dry cough. In some cases, you experience a blocked or runny nose, sore throat, muscle pain, and diarrhea. The test results relate to the identification of SARS-CoV-2 nucleocapsid antigen. The antigen is generally detectable in upper respiratory tract samples or lower respiratory tract samples during the acute phase of infection. A positive test result suggests SARS-CoV-2 infection, but further testing by a doctor is needed to confirm infection. Negative results do not completely rule out SARS-CoV-2 infection.

Negative results should be confirmed by a doctor, especially if symptoms of COVID-19 are present. People who are unable to understand the instructions for use or who are unable to carry out the test themselves should only carry out the test under supervision and with outside help.

This test is based on gold colloidal immunochromatography. During the test, samples are applied to the test cards. If the sample contains the SARS-CoV-2 antigen, the antigen binds to the SARS-CoV-2 antibody. After the sample is applied to the test strip, the complex moves along the nitrocellulose membrane to the end of the absorbent paper. When passing the test line (line T, coated with another SARS-CoV-2 antibody), the complex of SARS-CoV-2 antibody is bound to the test line and shows a red line. When passing line C, a control antibody is bound so that a red line appears.

II. Purpose of the Test

The objective of this study is to evaluate and validate the performance of SARS-CoV-2 antigen test devices by comparison to a reference method, Polymerase Chain Reaction (PCR), assay at a third-party laboratory. The purpose of the study is verification the diagnostic specificity and diagnostic sensitivity of the in vitro diagnostic medical device: SARS-CoV-2 Antigen Test Kit (Colloidal Gold) (hereinafter referred to as "to-be evaluated reagent") for defined specimen type intended to be used during testing procedure by the end-user. The rapid tests are manufactured by **Anbio (Xiamen) Biotechnology Co., Ltd.** The comparator method should be one of the more sensitive RT-PCR assays authorized by FDA and CE.

1. Clinical Sample Selection

420 Samples obtained from travelers including 205 European travelers were investigated for the study. 212 Positive samples and 208 Negative samples were tested respectively.

(1) Inclusion Criteria:

A. Samples from travelers including European Travelers with traceable patients' information

B. The sample should be with clearly recorded source, all samples should have corresponding basic clinical information, including: sample number, gender, age and clinical diagnosis information. The clinical diagnosis information includes asymptomatic or with suspected symptoms of SARS-CoV-2. The collection and treatment of samples are in accordance with the reagent specification or relevant regulations.

(2) Exclusion Criteria:

If the following conditions occur, the test samples will be directly excluded/rejected:

A. Samples with human factors that cannot complete the test process (such as samples that are contaminated during the test operation).

B. The test sample contaminated with bacteria or/and nosebleeds.

C. The test sample gone through too many freeze-thaw cycles during storage.

D. The test sample not kept under the conditions required for storage or testing.

E. Test samples that cannot use PCR reagents to generate control signals.

F. Samples without clear medical information

G. Samples without traceable patient information

(3) QTY. of Samples:

212 Positive samples and 208 Negative samples were used during the study to compare the Antigen test result with RT-PCR results.

2. Intended Use

The COVID-19 antigen rapid test is a colloidal gold immunochromatography test for the qualitative detection of nucleocapsid antigens from SARS-CoV-2 in human saliva. It is suitable for use by people who are suspected of having a COVID-19 disease. Suitable for users over 18 years of age. Children and adolescents under the age of 18 should only take the test under adult supervision.

The new coronaviruses belong to the genus β-corona viruses. The disease they cause, COVID-19, is an acute infectious disease of the respiratory tract. Currently, people infected with the novel coronavirus are the main source of infection, but asymptomatically infected people can also be infectious. The incubation period is 1 to 14 days, usually 3 to 7 days.

The main symptoms are fever, tiredness and a dry cough. In some cases, you experience a blocked or runny nose, sore throat, muscle pain, and diarrhea.

The test results relate to the identification of SARS-CoV-2 nucleocapsid antigen. The antigen is generally detectable in upper respiratory tract samples or lower respiratory tract samples during the acute phase of infection. A positive test result suggests SARS-CoV-2 infection, but further testing by a doctor is needed to confirm infection. Negative results do not completely rule out SARS-CoV-2 infection. Negative results should be confirmed by a doctor, especially if symptoms of COVID-19 are present.

People who are unable to understand the instructions for use or who are unable to carry out the test themselves should only carry out the test under supervision and with outside help.

3. Principle of the Product Test

The double antibody sandwich method is adopted for this product to implement determination in the form of solid phase immunochromatography. The sample to be tested diffuses upward by capillary force at the sampling end, and when passing by the marker pad, the SARS-CoV-2 antigen in the sample is combined with the antibody on the marker pad to form a colloidal gold antibody-antigen complex. The complex continues to spread with the sample to reach the nitrocellulose membrane and is intercepted by the T- line (test line) coated with antibody, and the complex is captured to form an immune complex of colloidal gold antibody conjugates-antigen-coating antibody. The remaining colloidal gold conjugates continue to ascend and are combined with C-line (quality control line), indicating completion of the reaction.

4. Product Performance Parameters

Item No.	Parameters	Standard
1	Clinical Performance	**Sensitivity** 99.06% (95% CI:96.63%~99.89%) **Specificity** 100% (95% CI: 98.24%~100%) **Accuracy** 99.52% (95% CI: 98.29%~99.94%)
2	Limit of Detection	250 $TCID_{50}$/ml. Limit of Detection (LoD)
3	Hook Effect	No high dose hook effect was observed when tested with up to a concentration of 1.0 x $10^5 TCID_{50}$/mL of Gamma-inactivated SARS-CoV-2 virus with the Anbio Rapid COVID-19 Antigen Test (Colloidal Gold).
4	Cross reaction	No cross-activity or interference was seen.
5	Interference Response	No cross-activity or interference was seen.

5. Material

The test kit consists of Test Cassette, Saliva collection device (with 1 mL Extraction Solution), Funnel, Disposable pipette and Instructions for Use.

(1) The Test Cassette consists of the card housing and test strip. Test strip contains a sample pad, glass fiber (Colloidal gold labeled mouse anti-SARS-CoV-2 monoclonal antibody, Colloidal gold-labeled rabbit IgG), nitrocellulose (NC) membrane, The T-line (test line) of nitrocellulose membrane is coated with mouse anti-SARS-CoV-2 monoclonal antibody, the C-line (quality control line) is coated with goat anti-rabbit IgG , absorbent paper and PVC plate.
(2) Extraction solution: the main component is phosphate buffer (PBS).
(3) Saliva collection device and Funnel: It is used for sample collection.
(4) Disposable pipette: It is used for droppng sample.

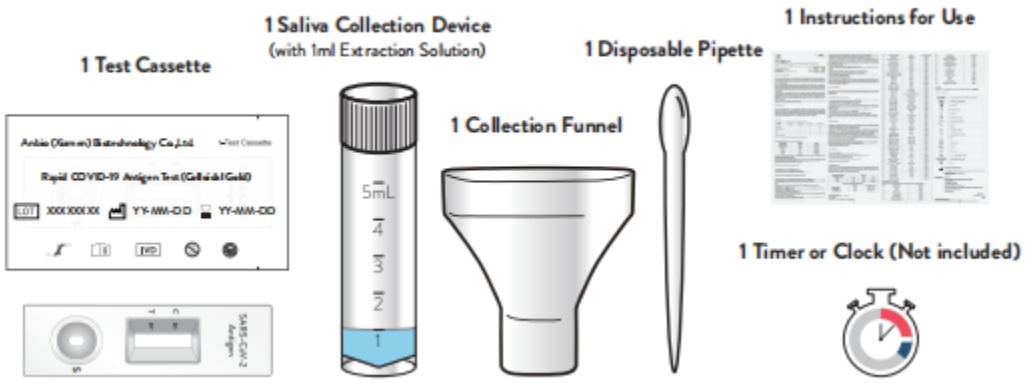
6. Test Procedure

Sample collecting

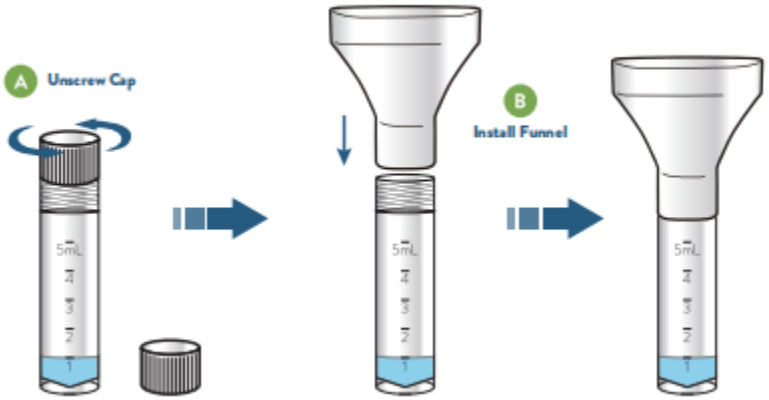
Type: Saliva sample

(1) Check the expiration date on the box. Do not use if the kit is Expired.

(2) Ensure that the kit is at Room temperature before use. Open the box and remove 1 each of the components shown below to perform a single test. Do not open individual components until instructed.



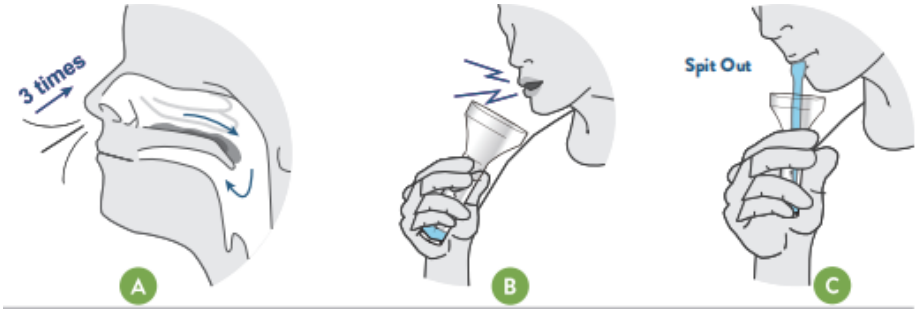
(3) Unscrew the cap of the collection tube and Install the collection Funnel in the collection tube.



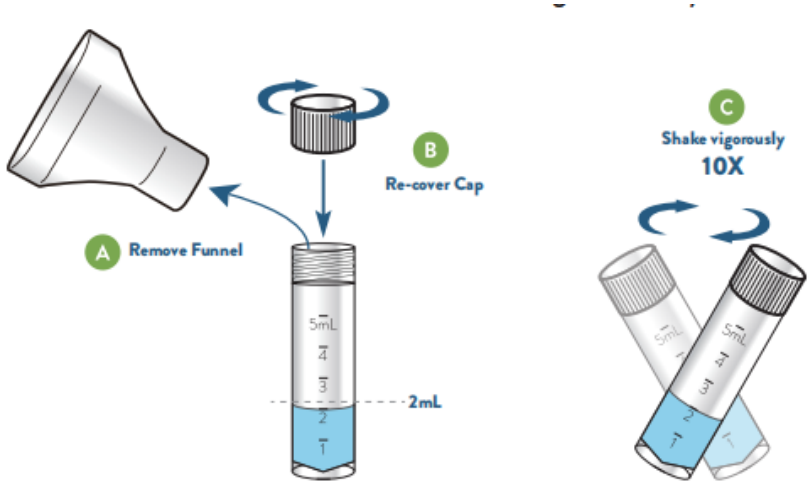
(4) Clear your throat and drain mucus from the back of your Nose and Throat at least **3** times.

(5) Lift the specimen collection funnel close to the mouth. Spit out the saliva with snorted nasal mucus into specimen collection tube.

Collect about **1mL** that is to the position of the graduation mark **2mL** of the collection tube.

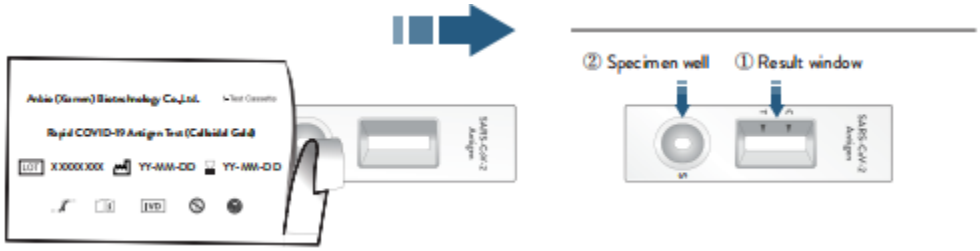


(6) Remove the collection funnel and Re-cover the Cap of the collection tube. Then shake the collection tube vigorously at least **10** times to mix the saliva and extraction reagents evenly.



Test procedure

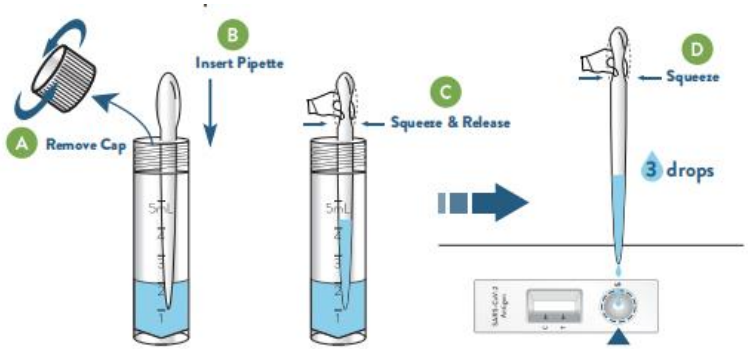
(7) Remove the Test Cassette from its protective package and place on a well-lit, flflat surface.



(8) Check liquid for bubbles of the extraction tube. Wait for any bubbles to disappear as they can lead to inaccurate results. Carefully Remove the white Cap on the Extraction Tube.

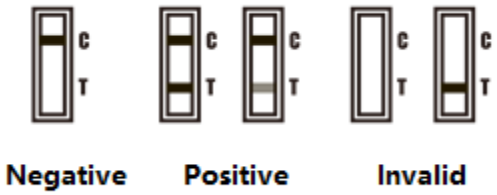
(9) Take the saliva sample with the disposable pipette.

(10) Squeeze 3 drops of liquid from the disposable pipette into the well (S) on the Test Cassette and start the time measurement. Secure white cap back on Tube and wait 15 minutes.



(11) Keep Test Cassette flflat on table. Read the result at 15 minutes. Do not read the result earlier than 15 minutes or after **20**minutes.

Interpretation of Results



(This picture is for reference only, and the real product should prevail)

(1) Negative (-):
Only line C is colored (see figure above), which indicates that the sample does not contain any SARS-CoV-2 antigen.

(2) Positive (+):
Colorings can be seen on both line C and line T (see figure above), which indicates that the sample contains SARS-CoV-2 antigen.

(3) Invalid:
No coloration can be seen on line C (see figure above). The test is invalid or an application error has occurred. Repeat the test with a new test cassette.

III. Test Management

1. Overview of Management Structure

This clinical trial was conducted by **PacGenomics Clinical Genetics laboratory**. As the applicant, **Anbio (Xiamen) Biotechnology Co., Ltd.** was responsible for communication and contact during the clinical trial.

2. Quality Control of the Laboratory

(1) All investigators participating in this clinical trial passed the qualification examination and had professional background and capacity related to clinical trial. Before clinical trial, all investigators had enough understanding and knowledge for specific contents of the clinical trial protocol and all indicators through training.
(2) The quality control of the laboratory met the requirements of quality control of clinical laboratory to ensure the standardization of experimental operating instructions.
(3) Quality control before the analysis: Check whether sample collection and processing meet the requirements, and whether sample number and other information are correct.
(4) Regularly inspect the implementation and completion of the clinical trial. Check integrity and accuracy of clinical sample information and verify test results.

3. Statistics and Data Management

(1) All selected cases were filled in clinical outcome summary, including subject sample number, age, gender, etc. Experimenters filled the test results of both the Reference Kit and the test kit in the clinical outcome summary.
(2) After finishing data entry, main investigators, experimenters and applicant reviewed the data together and locked data without any doubt.
(3) The clinical outcome summary was then submitted to analysts for statistics and analysis. The obtained statics and analysis results were filled in corresponding parts of clinical report.

4. Data Preservation

The test unit and the applicant kept one copy of clinical trial data respectively, including the following: Clinical Test Agreement, Clinical Test Protocol, Ethics Committee Instructions, Clinical Test Report (Test unit reports), General Report on Clinical Test, and Clinical Outcome Summary.

5. Problems Found During Investigation and Measures

In clinical trials, when a small number of samples are tested, the results of the Reference Kit and the Test Kit may be inconsistent. In this case, the clinical quantitative data of the samples involved in the test and the other common clinical trial reagents produced with the same principle are used for re-test.

6. Ethics

PacGenomics Clinical Genetics laboratory will uphold the highest level of ethical practice by respecting potential and enrolled subjects at all times. This includes:

(1) Highlighting all potential risks and benefits of participating in the study prior to patient enrollment and obtaining informed consent.
(2) Respecting participant privacy and keeping their personal information confidential.
(3) Respecting the participant's right to withdraw from the study without penalty.
(4) Answering any questions and addressing any concerns regarding the study.
(5) Alerting the participant if results from comparator method are abnormal.

7. Financing and Insurance Financing

The financial aspects of this study are documented separately in a confidential agreement between Anbio and principal investigators.

IV. Test Design

1. Description of overall test design and protocol

A total of 420 samples obtained from travelers including 205 European travelers were tested during the study. All samples are confirmed with a EUA test with high sensitivity and reverse transcription polymerase chain reaction (RT-PCR). PCR from the FDA SARS-CoV-2 Reference Panel available will be preferred as a comparator method. Additional information will be provided with the samples including: the date of onset of symptoms; the date of sample testing positive or negative by PCR. the SARS-CoV-2 molecular testing details, including specific type of test used and relevant medical history. The specimen should be used to test immediately after collection and should not be frozen and thawed. A blind and randomized method are used for comparison with reliable. All samples are masked with sample ID and randomly mixed. The operators performing Antigen Test are different from the operators performing RT-PCR to ensure pervious bias is not introduced.

2. Specimen Information

Specimen Type: Saliva

Collection Site:: Hollywood Hills Women's Medical Group

Collection Date: 3/25/2021-4/17/2021

Site Address： 9201 W Sunset Blvd, Suite 401, West Hollywood, CA 90069

QTY. of Collections： 420

Collector: Dr. Karen Lapesarde

3. Verified Device Information

Product Name:	Rapid COVID-19 Antigen Test (Colloidal Gold) / Saliva
Product Ref. No.:	A6061269
Packing Specification:	20 Test/Kit
Product Lot No.:	2021261313
Production Date:	2/28/2021
Expiration Date:	2/27/2023
IFU Version:	Rev V2.0
Manufacturer:	Anbio (Xiamen) Biotechnology Co., Ltd.
Address:	No.2016, Wengjiao West Road, Xinyang Street, Haicang District, Xiamen, Fujian, China
Tel	+86 592 6312399
Email:	info@anbio.com

4. RT-PCR Device Information for Control Test

RT-PCR Kit:	TaqPath™ RT-PCR
Product Ref. No.:	A47817
Packing Specification:	1000kits/box
Product Lot No.:	2008052
Production Date:	9/11/2020
Expiration Date:	8/13/2021
Certification:	CE/FDA-EUA
Manufacturer:	Life Technologies Corporation
Address:	6055 Sunol Blvd, Pleasanton, CA 94566

Extraction Kits:	Applied Biosystems MagMax Viral/Pathogen
Product Ref. No.:	A42359
Packing Specification:	550ml/Bottle
Product Lot No.:	2010112
Production Date:	10/11/2020
Expiration Date:	10/11/2021
Certification:	CE/FDA-EUA
Manufacturer:	Life Technologies Corporation
Address:	2130 Woodward st, Austin, TX 78744

PCR Analyze:	Applied Biosystems 7500 Fast Dx Real-Time PCR System
Manufacturer	Thermo Fisher Scientific
Address:	2130 Woodward st, Austin, TX 78744
Serial Number of PCR Analyzer:	275032390
Calibration Date:	01/18/2021
Maintenance Due Date:	01/18/2022

5. Laboratory Site Information

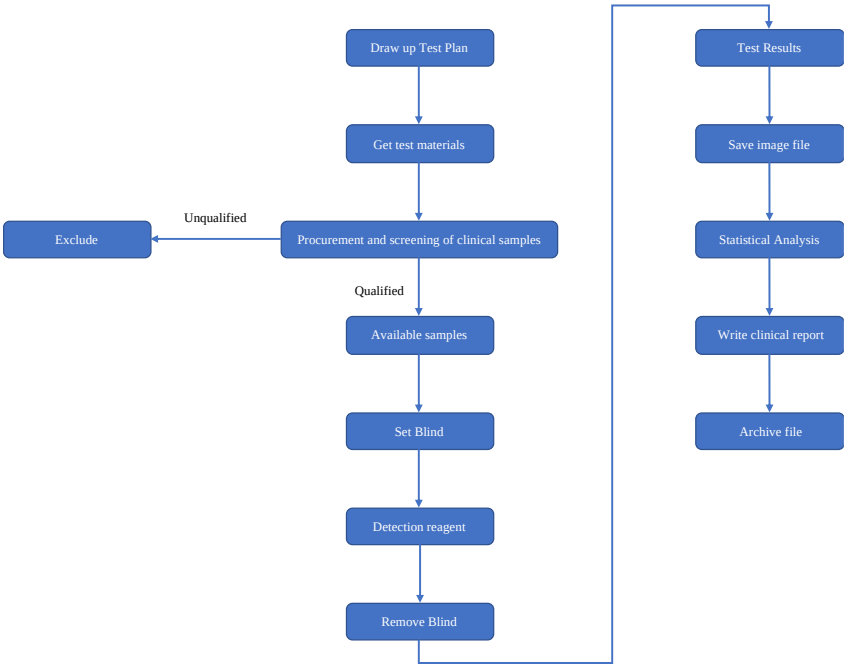
Testing Site:	PacGenomics Clinical Genetics laboratory
Registration No:	NPI1841715471
CLIA ID No.	05D2047289
Address:	28222 Agoura Road, Suite 200/201, Agoura Hills, CA 91301, USA
Tel	+1 818-597-1938

Email: shuanghou@pacgenomics.com

Investigator: Dr. Hua Li

Audit: Dr. Lian Liu

6. Test Procedure



7. Data Collection

All the testing data has been listed in datasheet contain matched candidate and reference testing results. (Raw data will be provided for the manufacturer and attached to the final reports as supportive evidence).

8. Statistical Analysis Method of Clinical Evaluation Data

Use the following formula for statistical analysis:

Rapid COVID-19 Antigen Test (Colloidal Gold) / Saliva Results	**RT-PCR Results**		
	Positive	**Negative**	**Total**
Positive	A	B	A+B
Negative	C	D	C+D
Total	A+C	B+D	A+B+C+D
Positive agreement (calculated diagnostic sensitivity)		[A /(A+C)] ×100%	
Negative agreement (calculated diagnostic specificity)		[D/(B+D)] ×100%	
Overall accuracy		[(A+D)/(A+B+C+D)] ×100%	
95% CI		Normal approximation	

V. Result Reading and Interpretation

According to the Instruction for use interoperated, RDT Antigen results were read after 15 minutes. Any shade in **T** line is considered to be the positive. Results were also confirmed with the one resulted from the Real-time PCR testing.

Total 420 samples were tested. 2 sample were tested Negative by using Antigen test kits, while the PCR tests were Positive. Please see the following summary table:

Rapid COVID-19 Antigen Test (Colloidal Gold) / Saliva **Results**	**RT-PCR Results**		
	Positive	**Negative**	**Total**
Positive	210	0	210
Negative	2	208	210
Total	212	208	420
Sensitivity	99.06% (95% CI:96.63%~99.89%)		
Specificity	100% (95% CI:98.24%~100%)		
Accuracy	99.52% (95% CI: 98.29%~99.94%)		

Additionally, sensitivity (positive coincidence rate) withing following CT range representing different viral load will be presented and analyzed. Data should be analyzer separately for each indicated specimen type.

CT range	**CT ≤ 25**	**25 < CT ≤ 28**	**28< CT ≤31**	**31 < CT**	**All CT**
No. of cases (RT PCR)	113	21	36	42	212
No. of positive candidate test results	113	21	36	40	210
Positive coincidence rate	100%	100%	100%	95.24%	99.06%

VI. Conclusion

The results of **Anbio Rapid COVID-19 Antigen Test (Colloidal Gold) / Saliva** showed a good agreement with PCR and clinical results. The clinical sensitivity of Anbio Rapid COVID-19 Antigen Test is 99.06%, clinical specificity is 100%. In conclusion, the Anbio Rapid COVID-19 Antigen Test has good clinical performance, and meets the clinical requirement.

Authorization Review

CEO of Pacgenomics: Dr. Lian Liu

Medical Director of Pacgenomics: Dr. Hua Li

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.

Headquarters' location:

PacGenomics.

CLIA ID No.: 05D2047289

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Institution and Researchers Involved in Clinical Trials

Institution: PacGenomics Clinical Genetics laboratory			
Item.	**Researchers**	**Title**	**Responsibility**
1	Dr. Lian Liu	CEO	Manage and Maintain the qualification of Laboratory
2	Dr. Hua Li	Medical Director	Manage and supervise the clinical study
Institution: Hollywood Hills Women's Medical Group			
3	Dr.Karen Lapesarde	Medical Doctor	Collect samples

Appendix

Appendix A: Laboratory Certificate

Appendix B: Initial Approval Letter

Appendix C: Summary of Clinical Trial Test Results

Appendix D: Image of Verified Device

Appendix E: Image of Control Device

Appendix F: Image of Test Results