COV04S-IFU-001, Revision 3

REF COV04S

PCL COVID19 Ag Gold Saliva

Instructions for use

Please read the instructions carefully before performing the test. Follow the instructions, and do not modify the process. Strict adherence to the guidelines will avoid inaccurate re-sults and achieve optimal performance of PCL COVID19 Ag Gold Saliva.

Product name

PCL COVID19 Ag Gold Saliva

Intended use

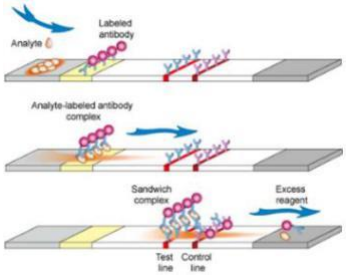
PCL COVID19 Ag Gold saliva is an in vitro diagnostic medical device based on the Immunochromatographic assay (ICA) principle for the qualitative detection of SARS-CoV-2 antigens in human saliva or naso-pharyngeal specimens. This test is used to detect antigens of the SARS-CoV-2 virus in people suspected of COVID-19. This product is in-tended exclusively for professional use in the laboratory or at the point-of-care.

Summary and explanation of the test

COVID-19 is a respiratory disease caused by a new type of coronavirus (SARS-CoV-2) first identified in December 2019 in Wuhan, China. Common signs of infection include, but are not limited to, respiratory symptoms, fever, cough, shortness of breath, reduced sense of smell or taste. In severe cases, the infection can cause pneumonia, severe acute respiratory syndrome, kidney failure, and death. Coronaviruses are a group of viruses that cause symptoms from the common cold to more severe illnesses such as Middle East Respiratory Syndrome (caused by MERS-CoV) and Severe Acute Respiratory Syndrome (caused by SARS-CoV).

Principle of the procedure

PCL COVID19 Ag Gold Saliva uses COVID19 antibodies, which are labeled with small gold particles and are attached to a nitrocellulose membrane near the sample hole of the test card (see also illustration below). After its application, capillary forces are pulling the sample from the sample hole to the test region of the device. When the liquid of the sample reaches the COVID19 antibodies, they detach from the membrane and are moved along the test card.



If the sample contains SARS-CoV-2 antigens (“analyte”), these bind to the labelled antibodies to form analyte-labeled antibody complexes. When these complexes reach the test line of the test card, they are retained on the test line by another set of COVID19 antibodies, which are immobilized on the nitrocellulose membrane. These so-called sandwich complexes appear as a color band on the test line. If the sample does not contain SARS-CoV-2 antigens, no sandwich com-plexes are formed and no color band appears on the test line.

Regardless of the presence or absence of SARS-CoV-2 antigens in the sample, a color band will appear on the control line of the test card. If no color band appears on the control line, the test card has not worked as intended.

Kit Components

Materials provided

Compo-nent	Description	Unit (Kit)			
		100	50	25	1
		Saliva	Saliva	Naso-pharyn-geal	Saliva
Test card	Test card with antibody coat-ing and built-in strip (pouch sealed)	100 ea.	50 ea.	25 ea.	1 ea.
Extraction buffer tube	Liquid reagent for sample ex-traction and de-velopment	4 buffer tubes, 15 mL ea. 100 empty tubes	50 buffer tubes, 500 µL ea.	25 buffer tubes, 500 µl ea.	1 buffer tube with 500 µL
Filter cap	Disposable lid for depositing a certain amount of sample on the test card	4 packs, 25 ea.	2 packs, 25 ea.	25 ea.	1 ea.
Applicator	Small paper funnel to trans-fer saliva to the Extraction buffer tube	100 ea.	50 ea.	---	1 ea.
IFU	Instructions for use	1 ea.	1 ea.	1 ea.	1 ea.

Required materials not included

- Timer or stopwatch
- Sterile swabs (in case of nasopharyngeal specimen)

Kit storage and stability

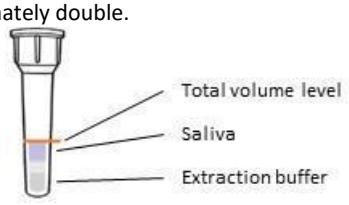
- PCL COVID19 Ag Gold saliva should be stored **at 2-30°C in a dry place**. When stored and handled as directed, the test cards and reagents are stable until expiration date indicated on kit labels.
- Test cards should be used immediately after opening the pouch.

Sample collection

Different specimen types have been validated with the PCL COVID19 Ag Gold Saliva test. Before the collection, do not eat, smoke or drink any beverages apart from water.

Saliva specimens

- When using the kit with 100 tests included, transfer 500 µL of Extraction buffer to an empty tube (included) for each sample to be tested.
- The person to be tested collects saliva in the mouth on the tip of the tongue for 30 seconds (approximately 0.5 mL); see also illustration below.
- Spit the collected saliva into the extraction buffer tube directly for immediate use. The applicator can be used to assist this step. By adding the saliva, the volume in the tube should approxi-mately double.



- Do not use stored specimens. Long-term storage may result in a signal decrease.
- Do not freeze the sample. Multiple freeze/ thaw cycles may re-sult in a signal decrease.

Nasopharyngeal swab specimens

- Insert the sampling swab through the nostril and gently push the swab into the posterior nasopharynx.

- Rotate the sampling swab three times
- Put the swab into the extraction buffer tube for immediate use.
- Do not freeze the sample. Multiple freeze/ thaw cycles may result in a signal decrease.

Warnings and precautions

- This product is intended for in vitro diagnostic use.
- This product is intended for single use.
- This product is intended for professional use.
- This product is intended for POCT use with human saliva and nasopharyngeal swab specimens.
- Assay should be performed as directed in the instructions for use to obtain accurate results.
- Do not use beyond the expiration date or damaged products.
- Do not use any other reagents that are not provided in this kit and do not mix components of different lots.
- This reagent can be stored at room temperature (15~25°C). Re-agents stored or samples collected at lower temperatures should be allowed to come to room temperature before use.
- Remove the test card from the pouch and use it as soon as pos-sible to avoid prolonged exposure to air. Prolonged exposure to air affects the test results.
- Follow laboratory test procedures for infectious diseases. Waste after use should be treated as infectious material and not dis-posed of randomly.
- Appropriate safety assurance procedures should be in place for infectious agents and materials.
- Wear gloves to handle samples and reagents.
- Do not suck the samples and reagents.
- Do not smoke, eat, drink, use cosmetic or touch contact lenses while handling the product.
- Spilled samples or reagents should be cleaned with disinfectants.
- Disinfect and dispose of all samples, reagents, and potential contaminants following applicable local regulations.

Preparation for use

⚠ *Reagents should be allowed to stand at room temperature for 20-30 minutes before testing. Do not use samples, which have been stored for prolonged times after collection.*

Assay procedure for saliva specimens

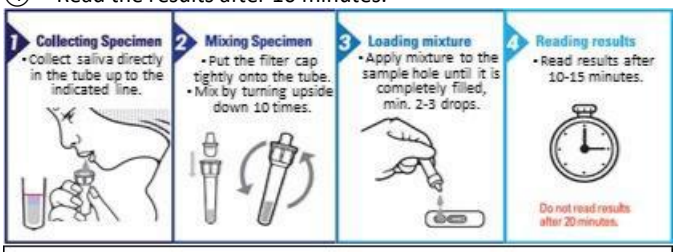
① Collect the sample as directed in the “Sample collection” section.

② Cover the tube with a filter cap and tighten the lid. Mix the contents by turning the tube upside down 10 times.

⚠ *Open the test card pouch just before use. If the pouch is left unused after opening, it may cause inaccurate results.*

③ Open the test card pouch and place the test card on a flat sur-face. Apply a few drops of the saliva extraction buffer mix into the sample hole of the test card. The sample hole should be al-most completely filled. Make sure not to use less than 2-3 drops.

④ Read the results after 10 minutes.



⚠ *Reading the test card later than 20 minutes after applying the sample diluent may give inaccurate results.*

Assay procedure for nasopharyngeal swab specimens

① Collect the sample as directed in the “Sample collection” section.

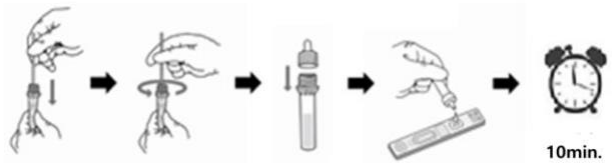
② Swirl the swab 10 times then remove it while squeezing the liq-uid from the swab.

③ Cover the tube with a filter cap and tighten the lid. Mix the contents by turning the tube upside down 10 times.

⚠ *Open the test card pouch just before use. If the pouch is left unused after opening, it may cause inaccurate results.*

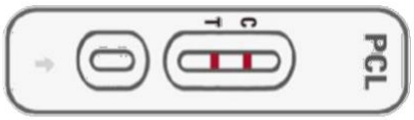
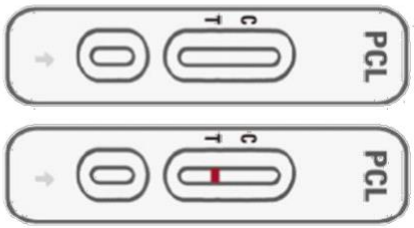
④ Open the test card pouch and place the test card on a flat sur-face. Apply a few drops of the saliva extraction buffer mix into the sample hole of the test card. The sample hole should be al-most completely filled. Make sure not to use less than 2-3 drops.

⑤ Read the results after 10 minutes.



⚠ *Reading the test card later than 20 minutes after applying the sample diluent may give inaccurate results.*

Interpretation of results

COVID-19 Ag non-reactive	
COVID-19 Ag reactive	
Invalid	

Using the test card can lead to three different results:

① If only one color band appears in the test region near the letter “C”, the result is valid and “non-reactive”, meaning no SARS-CoV-2 antigens could be detected.

② If a second color band appears in the test region near the letter “T”, the result is valid and “reactive”, meaning SARS-CoV-2 anti-gens were detected.

③ If no color band appears or if only one color band appears near the letter “T”, the result is invalid. In this case the result cannot be used, because the test did not work as intended. See section “Internal Control” for details.

Internal Control

The PCL COVID19 Ag Gold Saliva test contains a built-in internal control in the test card. A color band appearing in the control region (C) is designed as an internal control. The appearance of the control line confirms that sufficient flow has occurred, and that the test card is working normally. If the control line does not appear within 10 minutes, it is considered an error in the test result and it is recommended to test again with the same sample and a new device. If there is again no color band on the internal control line on the retest, con-tact the manufacturer or distributor.

External Controls

- External Positive and Negative controls may be used with the test kit. These controls provide additional quality control material to assess that the test reagents react as expected. Positive controls shall lead to "reactive" results and Negative controls shall lead to "non-reactive" results.
- Controls are recommended to be run once for each new kit lot.
- For external Positive control material, it is recommended to use "SARS-Related Coronavirus 2 (SARS-CoV-2) Culture Fluid (Heat Inactivated)" (Cat. No. #0810587CFHI) of "ZeptoMetrix" (USA).
- It is advised to divide "SARS-Related Coronavirus 2(SARS-CoV-2) Culture Fluid (Heat Inactivated)" (Cat. No. #0810587CFHI) into separate units each containing 15 µL and stored at -70℃ until use. Prior to use, allow the control material to stand at room temperature for at least 30 minutes and thaw completely.
- Prepare solutions for Positive controls following the instructions provided with the control material.
- For external Negative controls it is recommended to use the Ex-traction buffer included in the kit.
- Perform controls using the same procedure as used for patient specimens.
- If the kit controls do not perform as expected, do not report pa-tient results. Contact the manufacturer or distributor.

Limitations of the procedure

- The results of PCL COVID19 Ag Gold saliva should not be considered as absolute, and shall not be the sole basis for treatment or patient management. The infection should be confirmed by a specialist along with other experimental results, clinical symptoms, epidemiology, and additional clinical data.
- This kit detects both SARS-CoV and SARS-CoV-2, regardless of their viability. This kit does not differentiate between SARS-CoV and SARS-CoV-2.
- In the early stages of infection, low levels of antigen expression can result in non-reactive results.
- Due to the limitation of the assay methods, non-reactive results cannot entirely rule out the possibility of infection.
- This product can qualitatively detect SARS-CoV or SARS-CoV-2 antigens in human saliva or nasopharyngeal specimens and can-not determine the specific antigen quantity in the sample.

Performance characteristics

Limit of detection (LoD)

The LoD was determined using limiting dilutions of inactivated SARS-CoV-2 (ZeptoMetrix, #0810587CFHI) in two separate methods. The in-activated virus was spiked into the extraction buffer processed with a non-reactive saliva and Nasopharyngeal sample to have a concentra-tion of $TCID_{50}$ of 1.15 x 10^6/ml.

Each sample was serially 10-fold diluted and by testing in triplicate, a tentative LoD showing 100% (3/3) reactive rate was determined for each. For confirmation LoD study, 4 concentrations below the lowest concentration of the pre-test were tested in 20 replicates and a concentration showing over 100% (20/20) reactive results was deter-mined as the LoD of the PCL COVID19 Ag Gold Saliva for each.

- Saliva LoD: 1.44 x 10^3 $TCID_{50}$/ml
- Nasopharyngeal LoD: 1.44 x 10^3 $TCID_{50}$/ml

Cross-reactivity/ Microbial interference

Viruses/bacteria listed below were confirmed not to have cross-reac-tivity or cause interference with PCL COVID19 Ag Gold Saliva.

- Virus (10^5 $TCID_{50}$/mL): Adenovirus type 1, Adenovirus type 7, Coronavirus 229E, Coronavirus NL63, Coronavirus OC43, MERS-CoV, Cytomegalovirus, Influenza A H3N2, Influenza A H1N1, In-fluenza B, Enterovirus type 71, Parainfluenza type 1, Parainflu-enza type 2, Parainfluenza type 3, Parainfluenza type 4A, Measles virus, Human Metapneumovirus, RSV type A, RSV type B, Rhinovirus, Epstein Barr virus, Mumps virus and Corona-virus HKU1
- Bacteria (10^6 CFU/mL): B. pertussis, E. coli, H. influenzae, M. ca-tarrhalis, C. pneumoniae, L. pneumophila, M. pneumoniae, M. tuberculosis, N. meningitidis, P. aeruginosa, S. epidermidis, S. pneumoniae, S. pyogenes, S. salivarius and S. aureus

Endogenous interference

Potential interfering substances listed below were confirmed not to have a response with PCL COVID19 Ag Gold Saliva.

- Mucin (4 mg/mL), Human Blood (2%), 4-Acetamidophenol (10 mg/mL), Acetylsalicylic Acid (20 mg/mL), Chlorpheniramine (5 mg/mL), Diphenhydramine (5 mg/mL), Guaiacol glyceryl ether (20 mg/mL), Oxymetazoline (0.05 mg/mL), Phenylephrine (1 mg/mL), Fexofenadine (500 mg/mL), Amantadine (500 mg/mL), Ribavirin (500 mg/mL), Pseudoephedrine HCl (20 mg/mL), Ibuprofen (10 mg/mL) and Tamiflu (48 mg/mL), Naso GEL (5%), Chloraseptic (1.5 mg/mL), Cromolyn (15%), Zi-cam (5%), Homeopathic preparations (1:10 dilution), Sore Throat Phenol Spray (15%), Tobramycin (4 µg/mL), Mupirocin (10 mg/mL), Fluticasone Propionate (5%).

Clinical accuracy

The clinical performance of the PCL COVID19 Ag Gold Saliva in fresh saliva and nasopharyngeal swab specimens was evaluated in compar-ison to Real Time PCR results. Saliva and nasopharyngeal swab sam-ples for COVID-19 were collected from individuals diagnosed as positive or negative by RT-PCR testing.

- Saliva specimen

Positive percent agreement, PPA is 94.29% (95% CI: 80.84%-99.30%) and negative percent agreement, NPA is 100% (95% CI: 94.87%-100.00%) with PCL COVID19 Ag Gold Saliva.

PCL COVID19 Ag Gold Saliva	RT-PCR*		PPA (%)	NPA (%)
	Positive	Negative		
Positive	33	0	94.29	100
Negative	2	70		
Total	35	70		

*PowerChek™2019-nCoV Real-time PCR Kit: FDA-EUA, MFDS-EUA authorized and CE marked test

- Nasopharyngeal specimens

Positive percent agreement, PPA is 90.00% (95% CI: 85.00%-99.30%) and negative percent agreement, NPA is 100% (95% CI: 94.87%-100.00%) with PCL COVID19 Ag Gold Saliva.

PCL COVID19 Ag Gold Saliva	RT-PCR*		PPA (%)	NPA (%)
	Positive	Negative		
Positive	9	0	90	100
Negative	1	35		
Total	10	35		

*PowerChek™2019-nCoV Real-time PCR Kit: FDA-EUA, MFDS-EUA authorized and CE marked test

Key to symbols used			
REF	Catalog number	IVD	*In vitro* diagnostics medical device
LOT	Lot number		Consult instructions for use
Σ n	Sufficient for n tests		Do not reuse
30°C 2°C	Store at 2-30℃		
	Expiration Date		Caution
EC REP	European authorized representative	CE	Conformity European

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