

Evaluation of clinical performance

PCL COVID19 Ag Gold (COV 04S) for the *in vitro* qualitative detection of SARS-CoV-2 antigen in human saliva, nasal and nasopharyngeal swab specimens stored in VTM

Clinical institution: Chosun University Hospital (365, Pilmun-daero, Dong-gu, Gwangju Metropolitan City)

Principle investigator: Chosun University Hospital, Dong Min Kim

Clinical period: 2020/12/22 ~ 2020/12/31

IRB: CHOSUN 2020-11-042

1 Purpose

This study is intended to evaluate the clinical performance of PCL COVID19 Ag Gold (COV04S).

2 References

- MFDS guideline for IVD medical devices for high-risk infectious virus (No. 0629-02)
- CLSI EP12-A2_User Protocol for Evaluation of Qualitative Test Performance
- CLSI ILA21-A2_Clinical Evaluation of Immunoassays

3 Terms and definitions

- **Analyte:** Component represented in the name of a measurable quantity, the analyte is the particular component of interest to the patient
- **Sensitivity:** The probability that the device gives a positive result in the presence of the target marker.
- **True positive:** A specimen known to be positive for the target marker and correctly classified by the device
- **False negative:** A specimen known to be positive for the target marker and misclassified by the device
- **Specificity:** The probability that the device gives a negative result in the absence of the target marker
- **False positive:** A specimen known to be negative for the target marker and misclassified by the device
- **True negative:** A specimen known to be negative for the target marker and correctly classified by the device.

4 Product descriptions

4.1 Product name (model name)

PCL COVID19 Ag Gold (COV04S)

4.2 Classification

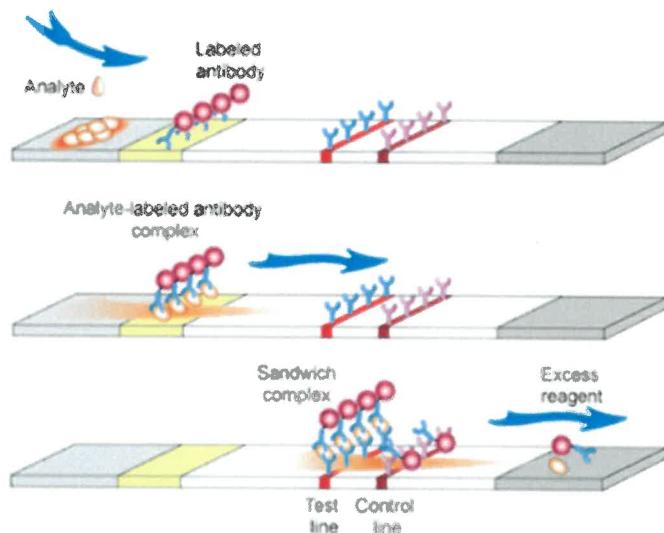
- IVDD Classification: General IVDs (Neither listed in the Annex II of the IVDD, nor self-testing device)
- MFDS Classification: Class 3 IVD (Reagents for infectious disease marker, K05030.1)

4.3 Intended use

PCL COVID19 Ag Gold 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, nasal or nasopharyngeal specimens. This test is used to detect antigens of the SARS-CoV-2 virus in people suspected of COVID 19. This product is intended exclusively for professional use in the laboratory or at the point-of-care.

4.4 Principles of procedure

PCL COVID19 Ag Gold detects the N protein (nucleocapsid protein) of SARS-CoV-2. The test 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 anti-bodies, 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 complexes 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.

5 Test descriptions

5.1 Test schedule and responsibilities

Test performed by (5 operators)	SY Hwang EK Bae MH Choi CM Kim DM Kim	Researcher (CUH)	2020.12.23 ~ 2020.12.24
Test site	Chosun University Hospital: 365 Pilmun-daero, Seonam-dong, Dong-gu, Gwangju, Korea		

5.2 Test materials

1) Test kit

Investigational kit			
Product name	PCL COVID19 Ag Gold		
REF	COV04S		
Manufacturer	PCL, Inc.		
Lot No.	2010S404	2010S406	
Expiration date	2022.10.09	2022.10.11	

Comparative kit	
Product Name	Real-Q 2019-nCoV Detection Kit
REF	BS7nCoV
Manufacturer	BioSewoom

2) Specimen

Saliva	No. of samples	Collection site	Testing site	RT-PCR test used for confirmation
Positive	120	SML	CUH	Real-Q 2019-nCoV Detection Kit
Negative	200	SML	CUH	Real-Q 2019-nCoV Detection Kit

Nasal swab	No. of samples	Collection site	Testing site	RT-PCR test used for confirmation
Positive	120	SML	CUH	Real-Q 2019-nCoV Detection Kit
Negative	200	SML	CUH	Real-Q 2019-nCoV Detection Kit

Nasopharyngeal swab	No. of samples	Collection site	Testing site	RT-PCR test used for confirmation
Positive	120	SML	CUH	Real-Q 2019-nCoV Detection Kit
Negative	200	SML	CUH	Real-Q 2019-nCoV Detection Kit

- SML: Samkwang Medical Laboratories, Seoul, Rep. of Korea
- CUH: Chosun University Hospital, Gwangju, Rep. of Korea

5.3 Specimen Provider/Specimen inclusion and exclusion criteria

Specimen provider inclusion criteria

- Patients suspected of respiratory infections due to symptoms (<10 days post symptom onset) such as coughing, phlegm, fever, etc. who have been identified with COVID-19 by RT-PCR method.
- Patients who are agreed to provide nasal, nasopharyngeal swab and saliva samples.

Specimen inclusion criteria

- Residual nasal, nasopharyngeal swab and saliva samples that will be discarded after the virus test and stored in VTM.
- Specimen guaranteed to be anonymized that have been completely removed personal identification
- Specimen stored frozen at -70°C in VTM.

Specimen provider exclusion criteria

- Patients with unknown COVID-19 infection

Specimen exclusion criteria

- Insufficient samples for testing
- Sample containing excessive amounts of blood
- Specimens suspected of microbial contamination
- Pooled sample
- Samples with broken containers or unlabeled samples
- Sample consisting of a different type of body fluid, other than nasal, nasopharyngeal and saliva samples stored in VTM.

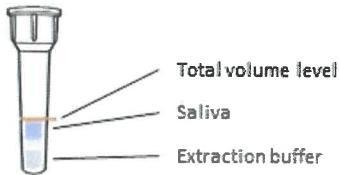
5.4 Test methods

1) Sample collection

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

Saliva specimen

- 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 approximately 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 result in a signal decrease.

Nasopharyngeal swab specimen

- Insert the sampling swab through the nostril and gently push the swab into the posterior nasopharynx.
- Rotate the sampling swab three times then slowly remove from the nostril.
- 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.

Nasal swab specimen

- Insert the sampling swab for less than one inch into the nostril (about 2 cm).
- Place the swab against the nasal wall and rotate the sampling swab five times. Then slowly remove the swab from the nostril.
- With the same swab, repeat the nasal swab specimen collection for the second nostril.

※ Caution: If the sampling swab stick breaks while sample collection, repeat the same procedure with a new swab.

- Once collected from both nostrils, 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.

2) Kit preparation

- 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.

3) 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 surface. Apply a few drops of the saliva extraction buffer mix into the sample hole of the test card. The sample hole should be almost 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.

4) Assay procedure for nasopharyngeal/nasal swab specimens

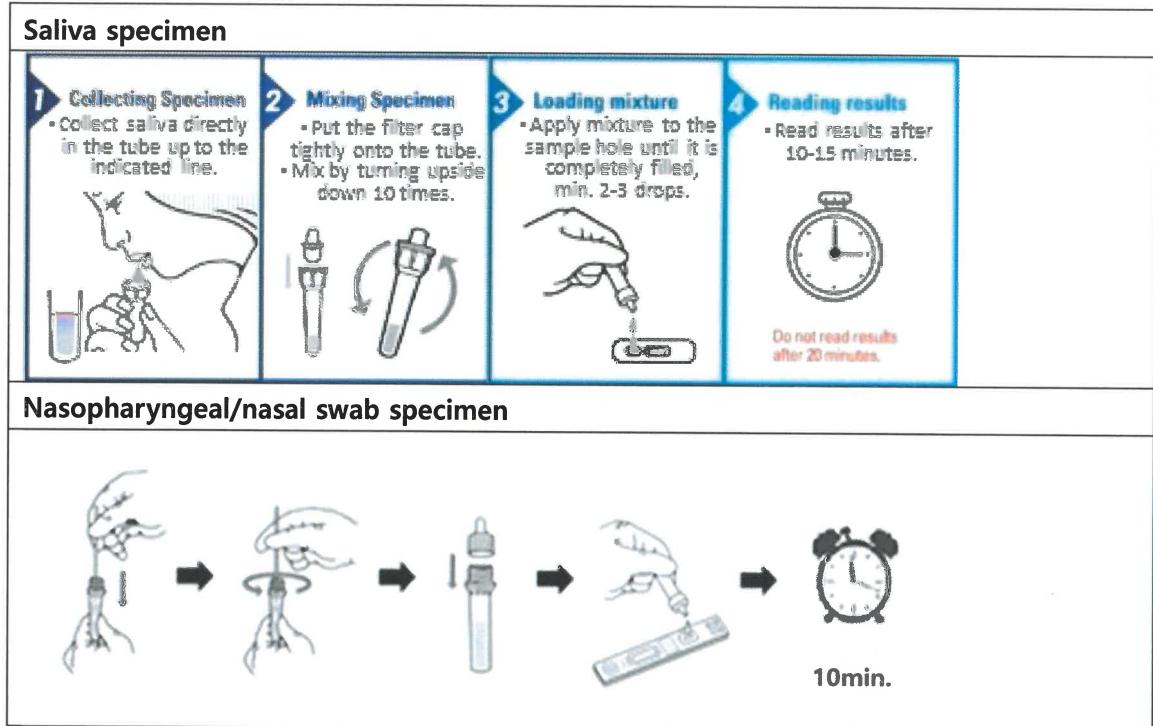
- ① Collect the sample as directed in the "Sample collection" section.
- ② Swirl the swab 10 times then remove it while squeezing the liquid from the swab.
- ③ Cover the tube with a filter cap and tighten the lid. Mix the contents by turning the tube upside down 10 times.

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

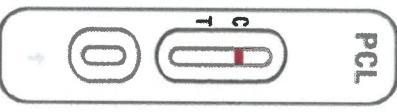
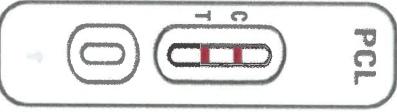
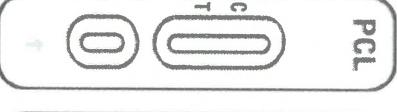
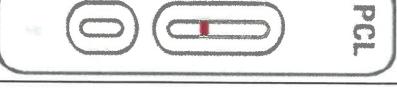
- ④ Open the test card pouch and place the test card on a flat surface. Apply a few drops of the saliva extraction buffer mix into the sample hole of the test card. The sample hole should be almost completely filled. Make sure not to use less than 2-3 drops.

- ⑤ Read the results after 10 minutes.

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



5) 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 antigens 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 can-not be used, because the test did not work as intended. See section "Internal Control" for details.

6) Data analysis

Percent agreement: Test results will be expressed in 2X2 table and analyzed as percent agreement with comparator RT-PCR testing results, retrospectively.

		RT-PCR assay	
		Positive	Negative
PCL COVID19 Ag Gold	Positive	A	B
	Negative	C	D

- Positive Percent Agreement (PPA) = A / (A + C)
- Negative Percent Agreement (NPA) = D / (B + D)

Internal Controls

- The PCL COVID19 Ag Gold 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, contact 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°C 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 Extraction 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 patient results. Contact the manufacturer or distributor.

7) Acceptance criteria

Clinical test results should have a Positive Percent Agreement (PPA) of at least 80% and a Negative Percent Agreement (NPA) of at least 90% compared to the confirmatory test device.

6 Test results

PCL COVID19 Ag Gold (NP swab result)	Comparator Method		
	Real-Q 2019-nCoV Detection Kit		
	(NP swab result)		
Positive	Positive	Negative	Total
Positive	110	0	110
Negative	10	200	210
Total	120	200	320
Positive Percent Agreement (PPA)	91.67 % (95% CI: 85.34% - 95.41%)		
Negative Percent Agreement (NPA)	100 % (95% CI: 98.12% - 100%)		

PCL COVID19 Ag Gold (saliva result)	Comparator Method		
	Real-Q 2019-nCoV Detection Kit		
	(NP swab result)		
Positive	Positive	Negative	Total
Positive	109	1	110
Negative	11	199	210
Total	120	200	320
Positive Percent Agreement (PPA)	90.83 % (95% CI: 84.33% - 94.8%)		
Negative Percent Agreement (NPA)	99.50 % (95% CI: 97.22% - 99.91%)		

PCL COVID19 Ag Gold (Nasal swab result)	Comparator Method		
	Real-Q 2019-nCoV Detection Kit		
	(NP swab result)		
Positive	Positive	Negative	Total
Positive	108	0	108
Negative	12	200	212
Total	120	200	320
Positive Percent Agreement (PPA)	90 % (95% CI: 83.33% - 94.19%)		
Negative Percent Agreement (NPA)	100 % (95% CI: 98.12% - 100%)		

7 Conclusion

A total of 960 blinded frozen samples (320 NP swabs, 320 saliva and 320 Nasal swabs) consisting of 360 positive (120 NP swabs, 120 saliva and 120 Nasal swabs) and 600 negative (200 NP swabs, 200 saliva and 200 Nasal swabs) specimens were tested to evaluate the sensitivity and specificity of the PCL COVID19 Ag Gold by five minimally trained operators in Chosun University Hospital under respective IRB approval. The

performance was evaluated retrospectively by comparing the results obtained with the PCL COVID19 Ag Gold (REF: COV04S) and the results obtained with COVID-19 RT-PCR (Real-Q 2019-nCoV Detection Kit: FDA-EUA authorized (BioSewoom)). In case of nasopharyngeal swab samples, the positive percent agreement (PPA) is 91.67 % (95% CI: 85.34% - 95.41%), and the negative percent agreement (NPA) is 100 % (95% CI: 98.12% - 100%). In case of saliva samples, the positive percent agreement (PPA) is 90.83 % (95% CI: 84.33% - 94.8%), and the negative percent agreement (NPA) is 99.50 % (95% CI: 97.22% - 99.91%). In case of nasal swab samples, the positive percent agreement (PPA) is 90 % (95% CI: 83.33% - 94.19%), and the negative percent agreement (NPA) is 100 % (95% CI: 98.12% - 100%).

8 Appendix

- Appendix 1. Raw data

1. Site: CUH (Chosun university hospital, Gwangju, Rep. of Korea), 5 Operators. According to IFU

