

A Synchronous Blind Test and Methodological Comparison Between Colloidal Gold Rapid Test vs RT-PCR For Sars-Cov-2 Detection

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ABSTRACT

Background: The current pandemic due to novel SARS-CoV-2 virus dramatically affected health care systems and public health worldwide. The present study aimed to evaluate two analytical methods, colloidal gold antigen rapid test vs reference PCR for the detection of SARS-CoV-2. The patients enrolled in the trial were admitted at the emergency department of a tertiary care hospital, with symptoms of suspected COVID-19 disease.

Methods: A total of 300 patients participated in the study. Patients' age, gender and result from the Colloidal antigen rapid test were recorded. PCR detection for SARS-CoV-2 was then applied, according to the manufacturer's instructions. Statistical analysis of all collected data was performed for sensitivity, specificity, ROC curves, PV+, PV- and Cohen's kappa coefficient. McNemar's chi-squared test and p-values were also tested.

Results: A p-value=0.045 from McNemar's chi-squared test for CI 95% was observed, so H0 marginally is not rejected. The sensitivity of colloidal gold antigen rapid test was 79%, the specificity 96%, PV+89%, PV-91% and the kappa coefficient=0.79 (>0.5) that correlates to substantial agreement according to Cohen's Kappa interpretation.

Conclusions: Through the methodological comparisons and according to WHO guidelines for the sensitivity, specificity and kappa coefficient that correlates to substantial agreement the Colloidal Gold Antigen Rapid Test for SARS-CoV-2 meet the needs of clinical test in the emergency unit playing an important role in the context of mass patient screening and screening in remote areas.

Keywords: Antigen Rapid Test, COVID-19, ECDC, kappa coefficient, PCR, ROC curves, SARS-CoV-2, sensitivity, specificity, WHO.

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I. INTRODUCTION

World Health Organization (WHO) declared in early March 2020 a pandemic [1] caused by the virus SARS-CoV-2 (severe acute respiratory syndrome coronavirus [2] responsible for the disease named COVID-19 [2]. The virus's genome size is 26-32 kb, and it is a single-stranded positive-polarity RNA molecule [3], [4].

SARS-CoV-2 genome encodes four types of structural proteins (spike S, envelope E, membrane M and nucleocapsid N proteins) and several non-structural ones [8].

Coronaviruses share the common characteristic to continuously evolve, as their genetic code changes during replication of their genome. Due to SARS-CoV-2 high transmissibility, several mutations have emerged worldwide caused by random genetic mutations or viral recombination.

WHO, ECDC (European Centre for Disease Prevention and Control) and CDC (Centers for Disease Control and Prevention) classified the most infectious mutations that observed worldwide and designated them as Variants of Concern (VOC) or Variants of Interest (VOI) [5]–[7].

However, as the virus continues to mutate, the effectiveness of its detection by RT-PCR and Antigen Rapid tests continues to increase. The Standard RT-PCR method for virus infection detection is applied to individual nasopharyngeal swabs and usually requires at least 4 hours of turnaround time, high cost, equipment, and trained laboratory staff. Usually, the genetic coding targets utilized for diagnostic purposes include identification, 2 or more, of Open Reading Frame 1 ab (ORF1ab), E gene, M gene and N gene [10]–[12]. Therefore, antigen rapid screening of potentially infected individuals, with high acceptable accuracy for SARS-CoV-2, is urgently needed for disease prevention and control management in widespread community transmission [9].

There are numerous rapid antigenic tests for SARS-CoV-2 diagnosis manufactured by health care companies. WHO accepts effective use of AT (Antigenic Tests) that share a minimum sensitivity of 80% and a minimum specificity of 97% [13], [14]. The rapid AT uses an immunochromatographic method, designed to detect the presence or absence of SARS-CoV-2 nucleocapsid protein in nasal fluid samples from suspected cases of COVID-19 disease.

The aim of the present study was to evaluate the clinical performance of the SARS-Cov-2 Antigen Rapid Test Kit (Colloidal Gold) produced by Hangzhou Bioer Technology CO, LTD for the accurate detection of SARS-CoV-2 antigen in human nasopharyngeal fluid. The aforementioned kit differs from numerous kits in the market, because it contains different SARS-CoV-2 monoclonal antibodies specific for different virus antigens, thus providing larger scale detection of the virus and its mutations. The population under study consisted of 300 male and female patients who were admitted at the Emergency Department of Nikea General Hospital “Agios Panteleimon,” Piraeus, Greece. The nasopharyngeal swab samples were blindly numbered in order to eliminate the possible impact of the subjective bias, as well as the subjects to reduce the selection bias. No duplicate samples enrolled in the research. The study was approved by the Hospital’s Ethics Committee and carried out according to the Declaration of Helsinki.

II. MATERIALS AND METHODOLOGY

For the evaluation of the COVID-19 Antigen Test Kit (colloidal gold method) with REF BSK03S1S, manufactured by HANGZHOU BIOER TECHNOLOGY CO., LTD., we

used the synchronous testing and methodological comparison of 300 samples with the Bosphore SARS-CoV-2/Respiratory Pathogens Panel kit v1 with REF ABSCR3, manufactured by Anatolia Geneworks.

COVID-19 Antigen Test Kit (colloidal gold method) and Unio Viral DNA-RNA Extraction Kit 600 were stored at room temperature. BiobaseVTM was stored at 8 °C while Bosphore SARS-CoV-2/Respiratory Pathogens Panel kit v1 was stored in controlled temperature freezer at -20 °C, see in the Table I.

The study was performed according to the guidelines of the Helsinki Declaration of ethical principles for medical research involving human subjects.

Hospital professional medical staff wearing personal protective equipment (gloves, face protection, and lab coats) while handling the kits, collected the nasopharyngeal swab samples in accordance with the sampling method for COVID-19 Antigen Test Kit (colloidal gold method) and Bosphore SARS-CoV-2/Respiratory Pathogens Panel kit v1 respectively. Specimens were collected and randomly numbered by the Director of the Emergency Department and sent to the Molecular Laboratory of the Hospital.

Specimens for COVID-19 Antigen Test Kit (colloidal gold method) were immediately tested after sampling while specimens for the Bosphore SARS-CoV-2/Respiratory Pathogens Panel kit v1 were stored at 4 °C and tested within 48 hours. For Quality Control of the assay, we used both Internal Controls, as well as External Controls (AccuPlex™ SARS-CoV-2 Reference Material 0505-0126, Milford, MA, USA).

A. COVID-19 Antigen Test Kit (Colloidal Gold Method): Testing Procedure

Nasopharyngeal samples were collected with the nasopharyngeal swab included in COVID-19 Antigen Test Kit (colloidal gold method) package. For every 23 samples a positive and negative control swab was tested by the kit. The positive swab was provided by the manufacturer HANGZHOU BIOER TECHNOLOGY CO., LTD., while a sterilized swab was used as a negative control.

First, the big lid of the COVID-19 Antigen Kit sample extraction tube was unscrewed and placed on a clean surface. For the specimen collection the COVID-19 Antigen Kit nasopharyngeal swab was inserted into one nostril of the patient up to 2.5 cm from the edge of the nostril. After the swab was rolled 5 times along the mucosa inside the nostril to ensure that both mucus and cells are collected, the process was repeated with the same swab for the other nostril to ensure that an adequate sample is collected from both nasal cavities. The swab was then removed from the nasal cavity and imported into the extraction tube.

TABLE I: COLLECTION AND TESTING KITS USED IN THE STUDY

Reagent Name	COVID-19 Antigen Test Kit (colloidal gold method)	Bosphore SARS-CoV-2/ Respiratory Pathogens Panel kit v1	Unio Viral DNA-RNA Extraction Kit 600	Disposable Virus Sampling Tube
Specification	25 tests/box	100 reactions/box	96 tests/box	30 test/box
Storage conditions	2 °C~30 °C	-20 °C		2 °C~35 °C
Company	Hangzhou bioer technology Co., Ltd.	Anatolia Geneworks	Anatolia Geneworks	BiobaseBiodustry (Shandong) Co., Ltd

Into the extraction tube the villi part of the sampled swab was rolled 5 times into the solution through the outer wall while the tube was squeezed with fingers to dissolve as much as possible of the potential viral antigen from swab to solution. After the swab was removed and discarded the big lid of the extraction tube was screwed.

Next, the COVID-19 Antigen Kit test card was extracted from the aluminum sealed package and placed on a clean, level surface. To complete the procedure, the dropper lid was unscrewed and 3 drops (approximately 80 μ L) of the treated into the extraction tube sample were added into the sample well of the test card. The result was read in the chromogenic zone between 15~20 minutes and recorded to specific file, see in Fig. 1.

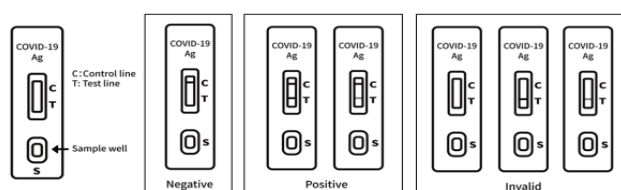


Fig. 1. COVID-19 Antigen Test Kit (colloidal gold method): result interpretation.

B. Real Time PCR: Test Procedure

For the real-time PCR testing, nasopharyngeal samples from the same patients were collected into the Disposable Virus Sampling Tube Kit manufactured by BiobaseBiodustry (Shandong) Co., Ltd. For the RNA extraction of the samples, Unio Viral DNA-RNA Extraction Kit 600 was used, manufactured by Anatolia Geneworks. Finally, for the detection of SARS-CoV-2 we used Bosphore SARS-CoV-2/ Respiratory Pathogens Panel kit v1, manufactured by Anatolia Geneworks.

Specimen collection for the PCR testing was made right after the specimen collection for the COVID-19 Antigen Kit testing was completed. The collection for both testing procedures was performed by the same hospital professional medical staff. The specimen was collected from nostrils with synthetic nasopharyngeal swab following the same procedure and then it was inserted and discarded into the medium of the Disposable Virus Sampling Tube Kit. Specimens were numbered and stored in cool place (4 °C) for up to 48 hours.

1) RNA Extraction with Unio Viral DNA-RNA Extraction Kit 600

Unio Viral DNA-RNA Extraction Kit 600 is compatible with Unio B2448 Extraction System manufactured by Anatolia Geneworks that uses magnetic bead method to extract nucleic acid from human samples. The kit consists of prefilled with all reagents and consumables cartridges. Proteinase K, Carrier RNA and sample need to be added to each cartridge before they are inserted into UNIO B2448 Extraction System as shown in Table II.

TABLE II: AMOUNT OF REAGENTS AND SAMPLE ADDED TO EACH CARTRIDGE

Reagents	Amount (μ L)
Proteinase K	20 μ L
Carrier RNA	10 μ L
Sample	600 μ L

All samples were placed into laminar and handled separately (no pooling). Before each sample was inserted into the cartridge, it was vortexed for 30 seconds. Then, the cartridges were placed into Unio B2448 where the Viral RNA Extraction program was selected as shown in Table III.

TABLE III: EXTRACTION PROGRAM SPECIFICATIONS

Kit Selection	VDR600
Kit Control and Sample Number	1-24 Samples
Sample Volume and Position	600 μ L-Direct in well
Elution Volume and Position	60 μ L-Direct in well

2) Real time RT PCR

For the SARS CoV-2 detection the Bosphore SARS-CoV-2/ Respiratory Pathogens Panel kit v1 by Anatolia Geneworks was used. The kit detects target gene ORF1ab and N for SARS-CoV-2 and uses the human endogenous nucleic acid sequence (RNase P) as an endogenous internal control (IC). The kit is compatible with Montania 4896 real-time PCR instrument. The LOD of the kit is 0,6 copies/ μ L for SARS-CoV-2.

All reagents were placed into a disinfected laminar and calibrated to room temperature. Then the Master Mix was prepared as shown to Table IV.

TABLE IV: MASTER MIX INGREDIENTS/ SAMPLE

PCR Master Mix 1	25,6 μ L
RT Mix	0,4 μ L
Sample and Positive/Negative Control	14 μ L
Total Volume/1 PCR tube	40 μ L

8-strip PCR tubes 0,2 mL were used for each PCR run. For each sample 26 μ L of Master Mix was inserted to each PCR tube. Then 14 μ L of extracted RNA were added using filtered tips.

8-strip PCR tubes filled with the Master Mix and the samples were sealed with the appropriate PCR tube caps and inserted into Montania 4896 real-time PCR instrument manufactured by Anatolia Geneworks. For each PCR run a positive and negative control was also tested.

The thermal protocol applied for the reaction is indicated in Table V:

TABLE V: THERMAL PROTOCOL

Stage	T °C	Time (min)	Cycles
Reverse Transcription	50	17:00 min	1
Initial denaturation	95	06:00 min	1
Denaturation	97	00:30 min	40
Annealing (Data Collection)	62	00:40 min	
Hold	32	02:00 min	1

C. Result Interpretation

After the thermal protocol was completed the results (positive/negative/invalid) were automatically interpreted by SLAN PCR program as shown in Table VI. The Threshold Value Ct is ≤ 30 for SARS-CoV-2 and ≤ 32 for the internal control (IC).

Statistical analysis was performed to calculate the positive and negative agreement rate respectively.

TABLE VI: RESULT INTERPRETATION

SARS-CoV-2 (FAM)	IC (Texas Red)	Result
+	+/-	Positive
-	+	Negative
-	-	Invalid

III. RESULTS

The human nasopharyngeal swab from 300 patients that were admitted at the Emergency Department of Nikaia General Hospital “Agios Panteleimon”, were collected according to the aforementioned methodology.

Our goal was to evaluate the clinical performance of the SARS-CoV-2 Colloidal Gold Antigen Test investigational device by comparison with the reference reagent Bosphore SARS-CoV-2 detection kit.

The samples were collected between March and June 2022.

Out of 300 patients, 139 were male (46.3%) and 160 females (53.3%). The mean age in male population was 50,55 years and 51,41 in female respectively. This data is presented in Table VII.


TABLE VII: DEMOGRAPHIC DATA OF THE STUDY POPULATION

Gender and Age Distribution Under the Study					
Gender			Age		
	Frequency	Percent	Mean	Min	Max
Male	139	46.3%	50.55	14	99
Female	160	53.3%	51.41	18	94
Not Recorded	1	0.003%	76.00	76	76
Total	300%		100%		

In Table VIII The consistency data analysis, based on statistical analysis method for clinical trial data are presented.

TABLE VIII: STATISTICAL ANALYSIS METHOD OF CLINICAL TRIAL DATA

Consistency data analysis			
Experimental Reagent Group (Colloidal Gold AT)	Reference Reagent Group (PCR)		Sum
	Positive	Negative	
Positive	α	b	$\alpha+b$
Negative	c	d	$c+d$
Sum	$\alpha+c$	$b+d$	$\alpha+b+c+d$
Sensitivity		$a/(\alpha+c)$	
Specificity		$d/(b+d)$	
Accuracy	$ACC/OPA=(\alpha+d)/(\alpha+b+c+d)\times 100\%$		
Kappa	$\frac{2(ad-bc)}{(\alpha+b)(b+d)+(\alpha+c)(c+d)}$		
95% CI	Normal approximation		

Use R 4.0.3  or the following formula for statistical analysis.

In Table IX are presented the Total Observations in 300 samples of the study.

Using our class table, provided by our data and by using the “epiR” package for conducting results, we calculated the following results for 0.95 confidence intervals (CI 95%) and using Agresti’s method.

Agresti’s confidence limits are calculated for test sensitivity, specificity, and positive and negative predictive value [27].

TABLE IX: POSITIVE AND NEGATIVE RESULTS BETWEEN COLLOIDAL GOLD ANTIGEN RAPID TEST AND REFERENCE PCR METHOD

	RAPID TEST		ROW TOTAL
	NEGATIVE	POSITIVE	
PCR TEST	203	7	210
	96.7%	3.3%	
	91.9%	8.9%	
	67.7%	2.3%	70%
	18	72	90
	20%	8%	
	8.1%	9.11%	
	6%	24%	30%
COLUMN	221	79	300
TOTAL	73.7%	26.3%	100%

TABLE X: CLASS TABLE PROVIDED BY OUR DATA

	Outcome +	Outcome -	Total
Test +	72	7	79
Test -	18	203	210
Total	79	221	300

TABLE XI: AGRESTI’S CONFIDENCE LIMITS

Apparent prevalence*	0.27 (0.22, 0.32)
True prevalence*	0.30 (0.25, 0.35)
Sensitivity*	0.79 (0.70, 0.87)
Specificity*	0.96 (0.93, 0.99)
Positive predictive value*	0.89 (0.83, 0.96)
Negative predictive value*	0.91 (0.87, 0.95)
Positive likelihood ratio	24.0 (12.44, 46.30)
Negative likelihood ratio	0.21 (0.14, 0.31)
False T+proportion for true D-*	0.04 (0.01, 0.07)
False T-proportion for true D+*	0.21 (0.13, 0.30)
False T+proportion for T+*	0.11 (0.04, 0.17)
False T-proportion for T-*	0.09 (0.05, 0.13)
Correctly classified proportion*	0.91 (0.88, 0.94)

*Agresti CIs

Point estimates and 95% CIs.

For Sensitivity-Specificity-PV+-PV-ROC curve: For producing our ROC curve a logistic regression model was fitted, with response variable the Colloidal gold Antigen Rapid test outcome (yes, no) and explanatory variable our golden standard test outcome.

The R package used for the plot was “ROCR” and below is Fig. 2.

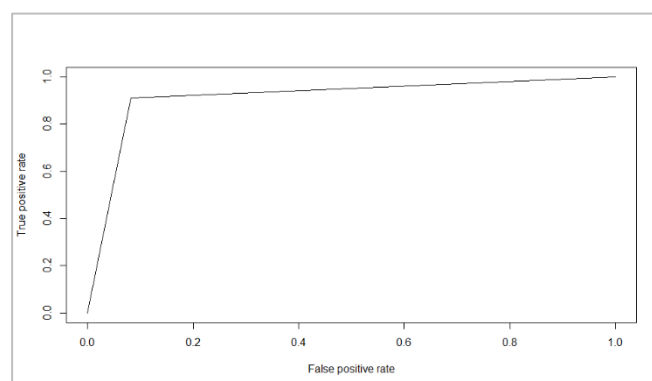


Fig. 2. ROCR for our study data.

Kappa coefficient (κ) is a measure of agreement beyond the level of agreement expected by chance alone. The observed agreement is the proportion of samples for which both methods (and observers) agree.

Common interpretations for the kappa statistic are as follows: <0.2 slight agreement, 0.2-0.4 fair agreement, 0.4–0.6 moderate agreement, 0.6-0.8 substantial agreement, >0.8 almost perfect agreement [26].

The z test statistic is. We have enough evidence to reject our null hypothesis towards the alternative that the kappa statistic is greater than zero. The proportion of agreement after chance has been excluded is 0.7944, meaning substantial agreement for CI 95% (95% CI, 0.68 to 0.91).

We conclude that, on the basis of this sample, there is substantial agreement between the two methods (i.e., Colloidal gold Antigen Rapid test and reference PCR method).

For our matched-paired data with a binary response (positive/negative) a test of marginal homogeneity has null hypothesis:

$$H_0: P(Y1 = 1) = P(Y2 = 1), \text{ or equivalently } H_0: \pi_{12} = \pi_{21}$$

Therefore, we conducted a McNemar's Test in order to examine if there is sufficient evidence in our data to determine that Colloidal gold Antigen Rapid Test is biased relative to reference PCR method.

McNemar's Chi-squared test:

$$X^2 = 4.84 \mid d.f. = 1 \mid p - \text{value} = 0.0278$$

McNemar's Chi-squared test with continuity correction:

$$X^2 = 4 \mid d.f. = 1 \mid p - \text{value} = 0.0455$$

The two-sided p-value in both coefficients (chi-squared and chi-squared with continuity correction) are below our level of confidence, *thus the evidence against marginal homogeneity is quite strong.*

IV. DISCUSSION

Numerous rapid antigenic tests for SARS-CoV-2 diagnosis have already been applied and massively used at the emergency departments of hospitals, but data evaluating their performance is poor [13]. According to World Health Organization recommendation of 80% sensitivity in antigenic rapid test is a crucial point, and still the question using AT in general population, and especially in asymptomatic, pre-symptomatic, or cases with symptoms resembling to COVID-19 disease [15]–[18].

The commercially available rapid tests based on serological techniques for SARS-CoV-2 have a sensitivity up to 100% but limited diagnostic value at the onset of COVID-19 disease, when the risk of viral transmission is higher [19], [20].

For accurate detection of SARS-CoV-2, molecules based on PCR methods have high sensitivity between 60–87% at the onset of COVID-19 disease [21].

The SARS-CoV-2 Antigen Rapid Test Kit (Colloidal Gold) uses the sandwich immunocapture method and colloidal gold immunochromatography to qualitatively determine the presence of SARS-CoV-2 antigens. SARS-CoV-2 antigens in the sample are bound by colloidal gold-labeled monoclonal anti-SARS-CoV-2 antibodies. Colloidal

gold nanoparticles are more biocompatible than other nanoparticles, thus with less environmental consequences [22], [23].

In the present study, a total of 300 nasopharyngeal swabs specimens were collected with no duplicate ones, between March to June 2022, (according to Sample size for studies with binary test outcome, Sample size for adequate sensitivity/specificity), [24].

The detection sensitivity was 79% and the specificity 96%, the PV+ was 89% and the PV- was 91%, presented in ROC curve, [25].

Cohen's kappa coefficient was found $\kappa=0.794$, fact that leads to the evidence of substantial agreement for the experimental Colloidal gold Rapid antigen test.

V. CONCLUSION

The study has performed a full analysis of the experimental reagents through methodological comparisons. The Cohen's kappa coefficient (κ) that is the statistic to measure inter-rater reliability (and also intra-rater reliability) for qualitative (categorical) items, in the present study was observed: $\kappa=0.79>0.5$ that correlates to substantial agreement according to Cohen's Kappa interpretation. Therefore, the results showed that Colloidal Gold Antigen Rapid Test for SARS-CoV-2 fulfilled the necessary criteria of clinical testing in the emergency unit and outpatient's department, playing an important role in the context of mass screenings and screening in remote areas.

AUTHORS' CONTRIBUTION

Antonia Mourtzikou and Dimitrios Tsiftsis conceived and designed the study, wrote the initial and final drafts of the article, and supervised the study. They both make equal contributions in this paper and are the principal investigators.

Antonia Mourtzikou, Panagiotis Koumpouras, Christina Seitopoulou and Elpida Toka carried out the reference PCR detection experiments, provided research scope and literature references.

Elpida Toka provided logistic support and funding acquisition.

Marilena Stamouli organized, statistically analyzed and interpreted the extracted data.

Maria Agrogianni, Nikolaos Kasimatis and Anthoula Dritsa collected the specimens, carried out the Colloidal AT, testing data collection details and contributed to the project administration.

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DISCLAIMER

This paper reflects the personal views of the stated authors. The funding has no role in the design of the study,

the collection, analysis, and interpretation of data and in writing the manuscript.

CONFLICT OF INTEREST

The authors declared no conflicts of interest.

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