Ocena testu antygenowego COVID19 Rapid FIA (PCL) do możliwego zastosowania w diagnostyce COVID-19 i badaniach przesiewowych

NIZP-PZH (29.05.2020r)

Szybkie testy diagnostyczne (RTD) wykrywające obecność antygenu (najczęściej białko N) wirusa SARS-CoV-2 pozwalają w czasie od 10 do 30 min. na wykrycie pacjenta z wysoką wiremią. WHO w dniu 8 kwietnia 2020 opublikowało opinię ekspertów, w której zdecydowanie zachęca do prowadzenia badań nad doskonaleniem testów antygenowych i oceny możliwości ich praktycznego zastosowania. Zgodnie z tym stanowiskiem, stosowanie szybkich testów na obecność antygenu SARS-CoV-2 (SARS2-Ag) w praktyce klinicznej nie mogło być rekomendowane ze względu na brak wystarczających danych piśmiennictwa, by takie rekomendacje przedstawić.[1]

Wykorzystanie testów antygenowych w innych, przebiegających z podobnymi objawami zakażeniach (grypa[2] i SARS[3]) pozwalają przyjąć założenie, że testy umożliwiające wykrycie antygenu SARS-CoV-2 znajdą zastosowanie do wczesnego wykrywania osób w ostrej fazie COVID-19. Dostępne nieliczne wciąż publikacje dotyczące wykorzystania testów RTD do wykrywania antygenu SARS-CoV-2 (SARS2-Ag) wskazują na znaczną rozbieżność czułości i swoistości stosowanych testów w granicach 50%-68% przy swoistości sięgającej do 100% z tym, że obserwowano wzrost czułości odpowiednio do 82%[4] do 98%[5], gdy pacjenci wykazywali w równoległym teście molekularnym RT-PCR wysoką wiremię (Ct<30). Problem określenia czułości, swoistości i możliwych zastosowań testów antygenowych jest tak istotny, że przygotowano wytyczne jak takie badania prowadzić, także retrospektywne[6].

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[1] Advice on the use of point-of-care immunodiagnostic tests for COVID-19 Scientific brief 8 April 2020 https://www.who.int/news-room/commentaries/detail/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-covid-19

[2] Bruning AHL, Leeflang MMG, Vos JMBW, Spijker R, de Jong MD, Wolthers KC, et al. Rapid Tests for Influenza, Respiratory Syncytial Virus, and Other Respiratory Viruses: A Systematic Review and Meta-analysis. Clin Infect Dis. 2017;65(6):1026–32. Available from: http://academic.oup.com/cid/article/65/6/1026/3829590/Rapid-Tests-for-Influenza--Respiratory-Syncytial

[3] I-Jung Liu, Pei-Jer Chen, Shiou-Hwei Yeh i wsp. Immunofluorescence Assay for Detection of the Nucleocapsid Antigen of the Severe Acute Respiratory Syndrome (SARS)-Associated Coronavirus in Cells Derived from Throat Wash Samples of Patients with SARS. JCM 2005;43; 2444-2448

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[5] Bo Diao, Kun Wen, Jian Chen i inni. Diagnosis of acute respiratory syndrome coronavirus 2 infection by detection of nucleocapsid protein (medRxiv preprint doi: https://doi.org/10.1101/2020.03.07.20032524)

[6] FIND https://www.finddx.org/wp-content/uploads/2020/04/20200421-COVID-Ag-RDT-Evaluation-Synopsis.pdf

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W dniu 25.04.2020r NIZP-PZH dokonało sprawdzenia laboratoryjnego testu diagnostycznego PCL COVID19 Rapid FIA wykrywającego antygen SARS-CoV-2 (SARS2-Ag). Do sprawdzenia użyto retrospektywnych próbek w postaci wymazów z gardła i nosogardła przekazanych do badania molekularnego na obecność SARS-CoV-2 oraz serii testu COV05-2003N405 (Załącznik 1). Poddany ocenie jakościowej test PCL COVID19 Rapid FIA wykazał się zgodnością 57% i 85% wyników odpowiednio wobec dodatnich i ujemnych wyników rtPCR. Najwyższą zgodność wyników dodatnich stwierdzono względem próbek o wysokiej wiremii (CT<26) oraz po wydłużeniu czasu inkubacji testu do 30 min (czułość 62%, swoistość 89%) w porównaniu do zaleceń producenta (10 min). Należy zaznaczyć, że badania nie przeprowadzono tak jak zaleca producent testu tj. bezpośrednio na materiale uzyskanym od pacjenta. Trzeba też wyraźnie podkreślić, że w przypadku zastosowania czasu inkubacji zalecanej przez producenta (10 minut) czułość testu była zdecydowanie niższa (15,4%), przy 100 % swoistości.

Wyniki sprawdzenia testu COVID19 Rapid FIA (PCL) z V Wojskowego Szpitala Klinicznego w Krakowie przy podobnej metodyce badań wykazały czułość (66%) i swoistość (93,2%) testu. Również w tym ośrodku stwierdzono współwystępowanie wyników dodatnich testu antygenowego z wysoką wiremią SARS-CoV-2 stwierdzoną u pacjentów w badaniu molekularnym (Ct 23-28). Podobne spostrzeżenia zawarto w piśmiennictwie dotyczącym ogólnego wykorzystania testów antygenowych w diagnostyce zakażeń ludzkimi koronawirusami (HCoV)[7]

Próbę praktycznego użycia szybkiego testu antygenowego COVID19 Rapid FIA (PCL) serii COV05-2003N405 i COV05-2003N401 podjęto w Szpitalu Zakaźnym w Warszawie. (Załącznik 3). Zbadano 266 próbek od pacjentów, u których równolegle przeprowadzono badanie RT-PCR. W tej grupie u 40 pacjentów wykryto RNA SARS-CoV-2 z czego u 16 uzyskano wynik pozytywny w szybkim teście na obecność antygenu (SARS2-Ag). Uzyskano również 5 fałszywie pozytywnych wyników SARS2-Ag, z czego 3 u pacjentów zakażonych HIV. Czułość testu oszacowano na 40% a swoistość na 98%. Potwierdzone RT-PCR wyniki dodatnie SARS2-Ag stwierdzono u pacjentów z podwyższoną wiremią (CT w zakresie 17-28). Natomiast wyniki SARS2-Ag, które uznano za fałszywie ujemne wykryto u pacjentów dla których wynik RT-PCR uzyskano dla Ct w zakresie 26-39, czyli częściowo w zakresie w którym w innych badaniach stwierdzono pozytywną korelację miedzy wynikiem SARS2-Ag a RT-PCR. W tych badaniach nie stwierdzono wzrostu czułości testu po przedłużeniu czasu inkubacji o dodatkowe 10 minut.

Ocenę przydatności testu podjęto też w warunkach Szpitalnego Oddziału Ratunkowego (SOR) Klinicznego Szpitala Uniwersyteckiego w Białymstoku na 265 pacjentach, od których jednoczasowo pobrano próbkę do badania testem RT-PCR (Załącznik 4). U wszystkich pacjentów nie stwierdzono obecności SARS2-Ag oraz nie wykazano obecność materiału genetycznego SARS-CoV-2 w teście RT-

[7] Michael J. Loeffelholz & Yi-Wei Tang (2020) Laboratory diagnosis of emerging human coronavirus infections – the state of the art, Emerging Microbes & Infections, 9:1, 747-756, DOI: 10.1080/22221751.2020.1745095

PCR, co zdaniem autorów badania pokazało że test COVID19 Rapid FIA (PCL) nie wykazał wyników fałszywie dodatnich w warunkach SOR, uzyskując 100% swoistości w zakresie wyników ujemnych.

W Uniwersyteckim Szpitalu Klinicznym w Bytomiu (SUM) przeprowadzono badanie SARS2-Ag u 10 pacjentów z objawami COVID-19 (z czego u 7 pacjentów potwierdzono zakażenie testem genetycznym oraz u 3 pacjentów nie potwierdzono zakażenia testem genetycznym) i 5 osób personelu medycznego. U wszystkich 15 badanych osób uzyskano testem antygenowym wyłącznie wyniki ujemne (Załącznik 5). Zaznaczyć należy, że w przypadku tych badań nie wykonano jednoczasowego testu RT-PCR. Wyniki SARS2-Ag odniesiono do wyniku testu RT-PCR wykonanego od 3 do 12 dni wcześniej. Uzyskany wynik może wskazywać na dość krótki okres utrzymywania się SARS2-Ag na poziomie wykrywalnym w metodzie COVID19 Rapid FIA (PCL). Aspekt ten wymaga dalszych badań.

Podobne wyniki uzyskano w ośrodku Wojskowego Instytutu Higieny i Epidemiologii (WIHiE) w Puławach, gdzie zbadano około 20 próbek od pacjentów ze wcześniej stwierdzonym zakażeniem SARS-CoV-2 na podstawie testu RT-PCR (Załącznik 6). W badaniu na obecność SARS2-Ag tych osób nie uzyskano ani jednego wyniku dodatniego, natomiast jednoczasowe z SARS2-Ag badanie szybkim testem kasetkowym na obecność przeciwciał dla SARS-CoV-2 wykazało obecność przeciwciał u 4 z tych pacjentów.

Powyższe wyniki i spostrzeżenia upoważniają do podsumowania wskazującego, że SARS2-Ag występować może jedynie w czasowo ograniczonym okresie zachorowania / zakażenia SARS-CoV-2 wynoszącym do kilku dni po wystąpieniu pierwszych objawów klinicznych. Ustalenie tego okresu wymaga dalszych badań polegających na ciągłej okresowej obserwacji obecności SARS2-Ag i odniesieniu jej jednoczasowo do wyniku RT-PCR oraz monitorowania wystąpienia przeciwciał swoistych dla SARS-CoV-2. Niestety dostępne nieliczne dane piśmiennictwa nie pozwalają na obiektywne wyznaczenie tego okresu. Można go wstępnie oszacować jedynie na podstawie wyników RT-PCR wskazujących na utrzymywanie się wysokiej wiremii (CT-poniżej 30).

Użyty do sprawdzeń zestaw diagnostyczny COVID19 Rapid FIA (PCL) posiada certyfikaty IVD i CE, co pozwala na jego stosowanie w diagnostyce in vitro – tj. do zastosowań w diagnostyce laboratoryjnej. Cechy tego zestawu stwierdzone na podstawie ww. raportów wskazują na jego niską zdolność do generowania wyników fałszywie ujemnych, oraz zdolność do wykrywania SARS2-Ag u pacjentów badanych wyłącznie w okresie podwyższonej wiremii (RT-PCR Ct poniżej 30), co jest zgodne z danymi piśmiennictwa (obecnie ograniczonego). Przy ocenie czułości i swoistości analitycznej aparatu należy uwzględniać udział pacjentów spełniających ww. warunki kliniczne. Niemniej jednak fakt ograniczenia czasowego występowania SARS2-Ag wpływać może znacząco na możliwości praktycznego zastosowania testu COVID19 Rapid FIA (PCL) zgodnie z jego właściwościami.

Należy jeszcze zwrócić uwagę, że zgodnie z obecnie obowiązującą definicją przypadku potwierdzonego COVID-19 zarówno w krajowym, jak i europejskim nadzorze epidemiologicznym testy antygenowe nie są uznawane za metodę dopuszczoną do laboratoryjnego potwierdzenia

przypadku. Jedynie badania RT-PCR pozwalają na dokonanie zgłoszenia przypadku potwierdzonego COVID-19 . Z tego względu każdy wynik dodatni testu antygenowego wymaga potwierdzenia w teście RT-PCR.

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- Dr hab. Grzegorz Juszczyk, Dyrektor NIZP-PZH.

Uwaga – treść załączników została przekazana przez Ministerstwo Zdrowia w jęz. angielskim do oceny przez Zespół ds. organizacji laboratoriów COVID.

Załącznik 1

Overall preliminary assessment of the PCL diagnostic test COVID19 Rapid FIA detecting antigen SARS-CoV-2 (SARS2-Ag)

National Institute of Public Health in Warsaw - 25.04.2020

The assessment included qualitative verification of the test based on 71 material samples:

1. clinical - throat and nasopharyngeal swab, taken for rtPCR testing for SARS-CoV-2 with a known molecular test result (n = 51),

2. from current research for which the molecular test result was not previously known (n = 12),

3. decimal dilutions of the suspension containing antigens of other coronaviruses of the alpha - NL63 and beta - OC43 groups (n = 8 [2x4]).

Clinical material from people with rtPCR test confirmed SARS-CoV-2 infection was tested, as well as from people with inconclusive and negative results. The results of the PCL COVID19 Rapid FIA test were read after 10, 15 and 30 min.

Results

During testing of 26 samples (positive in rtPCR), the presence of SARS-COV-2 virus antigen was detected in 15 (57%) cases using the PCL COVID19 Rapid FIA. It is worth noting, however, that a much greater convergence of the results of both tests was found in the case of extending the incubation in the cassette kit from 10 minutes (as recommended by the manufacturer) to 30 minutes. For this reason, the final result of the PCL COVID19 Rapid FIA test was taken after 30 min. In 20 samples with a negative rtPCR result, the presence of SARS2-Ag was found in 3 (15%), and in 16 samples with an inconclusive result, the presence of SARS2-Ag was found in 7 (44%). A positive PCL COVID19 Rapid FIA test result was not found in any of the samples containing 2 other than SARS-CoV-2 coronaviruses (NL63 and OC43).

Rating

The qualitatively evaluated PCL COVID19 Rapid FIA test showed compliance of 57% and 85%, respectively, against positive and negative rtPCR results. The highest concordance of positive results was found for samples with high viremia (CT <26). However, SARS2-Ag positive results were found among samples with negative rtPCR only when the result was read after 30 min. When reading the result after 10 minutes, 100% specificity, and after 30 minutes over 80% specificity of the PCL COVID19 Rapid FIA test with rtPCR was established. For samples with an inconclusive result, positive SARS2-Ag result was found mostly when the presence of the virus E gene was detected in the rtPCR test SARS-COV-2 (CT> 30).

Conclusions

The conducted tests show that the PCL COVID19 Rapid FIA test shows lower sensitivity than the PCR test. It cannot be excluded that this may be associated with the use of thawed clinical specimen samples. The PCL COVID19 Rapid FIA test can be useful for rapid, initial diagnosis of people infected with SARS-coV-2. However, its effectiveness will be limited to patients in a specific phase of infection, i.e. high viremia, probably only 3-4 days after obtaining certain positive rtPCR result. This is clearly seen in the results of tests of samples obtained twice at different periods of the disease from the same patient (samples 1791 and 3925). Relatively high share of positive results in the case of samples with an inconclusive rtPCR result may indicate the reactivity of the test with antigens of other coronaviruses, which have not been confirmed for NL63 and OC43, however. This however may indicate that the test will have the nature of a screening test and its results need to be confirmed by molecular test. The evaluation results indicate that the PCL COVID19 Rapid FIA test should be most useful for testing patients with signs of infection lasting several days.

Use in clinical diagnostics

A positive test result indicates the likelihood of SARS-CoV-2 infection, the patient should be referred to an infectious hospital to collect material for the rtPCR test necessary for case confirmation and, depending on the severity of the symptoms, hospitalized or placed in isolation.

A negative result does not exclude SARS-CoV-2 infection. If clinical symptoms suggesting SARS-COV-2 infection occur, molecular testing should be performed.

Appendix 1
Results of the confirmation tests

Tests carried out on 23 March 2020

No.	Isolate no.	PCR test	Ct	Confirmation test		Result	Antigen		
				E	RdRP		10’	15’	30’
1	1576	Genesig	17,61	15,82	18,90	Positive	P	P	
2	1577	Genesig	24,20	22,67	26,44	Positive	N	N	P
3	1580	Genesig	37,36	35,62	33,16	Positive	N	N	P
4	1585	Genesig	40,00	33,35	0,00	inconclusive	N	N	P
5	1588	Genesig	27,75	25,87	32,22	Positive	N	N	P
6	1589	Genesig	27,81	26,17	29,23	Positive	P	P	
7	1591	Genesig	34,73	32,57	0,00	inconclusive	N	N	P
8	1597	Genesig	32,92	31,77	0,00	inconclusive	N	N	N
9	1616	Genesig	19,53	34,69	0,00	inconclusive	N	N	P
10	1728	Genesig	29,52	29,48	34,76	Positive	N	N	N
11	1747	Genesig	40,00	19,28	0,00	inconclusive	N	N	P
12	1764	Genesig	37,33	35,19	36,14	inconclusive	P	P	

No.	Isolate no.	PCR test	Ct	Confirmation test		Result	Antigen		
				E	RdRP		10'	15'	30'
13	1775	Genesig	35,62	34,29	0,00	inconclusive	N	N	N
14	1805	Genesig	27,92	28,30	33,34	Positive	N	N	N
15	1811	Genesig	30,66	30,95	35,70	Positive	N	N	N
16	1831	Genesig	31,10	29,80	35,22	Positive	N	N	N
17	1864	Genesig	28,02	28,34	33,51	Positive	N	N	N
18	1915	Genesig	36,00	35,44	0,00	inconclusive	N	N	N
19	2041	Genesig	40,00	33,30	36,59	inconclusive	N	N	N
20	2198	Genesig	26,00	25,30	31,13	Positive	N	N	P
21	2210	Genesig	26,14	25,97	31,12	Positive	N	N	P
22	2297	Genesig	25,12	24,68	27,27	Positive	N	P	
23	2308	Genesig	26,13	26,25	29,86	Positive	N	N	P
24	2310	Genesig	27,87	28,08	32,02	Positive	N	N	P
25	2392	Genesig	36,17	35,12	37,01	Positive	N	N	N

Tests carried out on 23 March 2020

No.	Isolate no.	PCR test	Ct	Confirmation test		Result	Antigen		
				E	RdRP		10'	15'	30'
26	3071	SanSure	Orf 1ab 36,08 N cykl 34,80	36,15	37,24	Positive	N	N	N
27	3196	SanSure	Orf 1ab 29,36 N cykl26,20	33,05	34,10	Positive	N	N	P
28	3197	SanSure	Orf 1ab 37,83 N cykl 34,43	0,00	29,47	inconclusive	N	N	P
29	3245	SanSure	Orf 1ab 30,42 N cykl 30,31	31,42	0,00	inconclusive	N	N	N

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No.	Isolate no.	PCR test	Ct	Confirmation test		Result	Antigen		
				E	RdRP		10'	15'	30'
30	3463	SanSure	Orf 1ab 34,93 N cykl 33,76	30,92	0,00	inconclusive	N	N	N
31	3488	SanSure	Orf 1ab 31,14 N cykl 30,10	32,13	0,00	inconclusive	N	N	N
32	1822	Genesig 30.03.2020	0,00	0,00	0,00	Negative	N	N	N
33	3141	SanSure 11.04.2020	Orf 1ab 26,12 N cykl 22,82	26,06	0,00	inconclusive	N	N	N
34	3393	SanSure 13.04.2020	Orf 1ab 21,29, N 18,92	21,14	23,57	Positive	N	N	N
35	1826	Genesig 30.03.2020	25,90	26,51	31,00	Positive	N	N	N
36	2951	Genesig 09.04.2020	35,90	34,81	31,99	inconclusive	N	N	N
37	1825	Genesig 30.03.2020	31,96	32,53	36,90	Positive	N	N	N
38	2950	Genesig 09.04.2020	30,31	29,67	31,01	Positive	N	N	P
39	1791	Genesig 30.03.2020	28,53	29,05	33,81	Positive	N	N	N
40	3925	BGI 18.04.2020	17,57	19,59	21,63	Positive	P	P	P
41	1529	Genesig 27.03.2020	34,07	35,15	0,00	inconclusive	N	N	P
42	1724	Genesig 30.03.2020	21,00	21,62	26,88	Positive	N	N	P
43	4303	SanSure	0,00	0,00	0,00	Negative	N	N	P
44	4304	SanSure	0,00	0,00	0,00	Negative	N	N	N
45	4305	SanSure	0,00	0,00	0,00	Negative	N	N	N
46	4306	SanSure	0,00	0,00	0,00	Negative	N	N	N
47	4307	SanSure	0,00	0,00	0,00	Negative	N	N	P

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No.	Isolate no.	PCR test	Ct	Confirmation test		Result	Antigen		
				E	RdRP		10'	15'	30'
48	4308	SanSure	0,00	0,00	0,00	Negative	N	N	N
49	4309	SanSure	0,00	0,00	0,00	Negative	N	N	N
50	4310	SanSure	0,00	0,00	0,00	Negative	N	N	N
51	4311	SanSure	0,00	0,00	0,00	Negative	N	P	N
52	4399	LifeRiver	0,00	0,00	0,00	Negative	N	N	N
53	4401	LifeRiver	0,00	0,00	0,00	Negative	N	N	N
54	4403	LifeRiver	0,00	0,00	0,00	Negative	N	N	N
55	4404	LifeRiver	0,00	0,00	0,00	Negative	N	N	N
56	4406	LifeRiver	0,00	0,00	0,00	Negative	N	N	N
57	4407	LifeRiver	0,00	0,00	0,00	Negative	N	N	N
58	4411	LifeRiver	0,00	0,00	0,00	Negative	N	N	N
59	4412	LifeRiver	Orf 1ab 38,27 N (0) E (0)	0,00			N	N	N
60	4413	LifeRiver	Orf 1ab 36,41 N 34,90 E 35,14	0,00			N	N	N
61	4415	LifeRiver	Orf 1ab 16,71 N 16,23 E 16,02	17,19			P	P	P
62	4422	LifeRiver	Orf 1ab 36,53 N 35,19 E 34,70	0,00			N	N	N
63	4423	LifeRiver	0,00	0,00	0,00	Negative	N	N	N
64	NL63 1:10	n/a	n/a	n/a	n/a	n/a	N	N	N
65	NL63 1:100	n/a	n/a	n/a	n/a	n/a	N	N	N
66	NL63 1:1000	n/a	n/a	n/a	n/a	n/a	N	N	N

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No.	Isolate no.	PCR test	Ct	Confirmation test		Result	Antigen		
				E	RdRP		10'	15'	30'
67	NL63 1:10000	n/a	n/a	n/a	n/a	n/a	N	N	N
68	OC43 1:10	n/a	n/a	n/a	n/a	n/a	N	N	N
69	OC43 1:100	n/a	n/a	n/a	n/a	n/a	N	N	N
70	OC43 1:1000	n/a	n/a	n/a	n/a	n/a	N	N	N
71	OC43 1:10000	n/a	n/a	n/a	n/a	n/a	N	N	N

Subsequent result analysis

Results of tests carried out at NIZP-PZH (National Institute of Public Health) with the PCL COVID19 Rapid FIA kit on thawed samples (from the NIZP-PZH collection)

When testing 44 samples only with a clearly defined result in rtPCR.

READING AFTER 10 MINUTES (according to the manufacturer's instructions)

The result of the PCL COVID19 Rapid FIA kit	RT-PCR		sensitivity	specificity
	Positive result	Negative result		
Positive	4	0	15,4%	100%
Negative	22	18		
Together	26	18		

When testing 44 samples only with a clearly defined result in rtPCR.

READING AFTER 30 MINUTES (incubation extended by 20 minutes)

The result of the PCL COVID19 Rapid FIA kit	RT-PCR		sensitivity	specificity
	Positive result	Negative result		
Positive	15	3	62,1%	89,7%
Negative	11	15		
Together	26	18		

Załącznik 2

Fifth Military Clinical Hospital, Medical Analytics Department, Krakow, Poland

Kraków, 13 May 2020

Report on the validation process of the PCL COVID19 Ag Rapid FIA kit

We had at our disposal:

- test series number COV052003N401 - 3 packs, screw cap probes with extraction buffer, screw dropper
- test series number COV052003N402 - 1 pack, extraction buffer secured with foil blister after unpacking, press-in dropper

The test was performed in QUICK mode with incubation outside the device.

100 antigen determinations were carried out with a parallel RT-PCR determination.

In the case of 2 PCL COVID19 Ag determinations – test was rejected due to the lack of sample reading (*no sample or insufficient sample volume*), despite the correct dropping of the tested material (3 drops)

a) COVID19 was detected by RT PCR in 18 samples
- 4 were determined as positive based on the company's validation protocol with the detection of N gene; CT 19-38.5
- converging positive Ag - 12 results, including 1 N gene assay; CT 29

Values for which the PCL COVID19 Ag positive results were obtained were in the CT 23-28 range

b) false negative results for 6 determinations in the CT 30 - 38.5 range, with three determinations only regarding the detection of the N gene
c) for 6 samples, the RT PCR result was negative, with a positive PCL COVID19 Ag result, without COVID19 diagnosis, patients in further diagnostics, 2 of them with known interstitial pneumonia

The sensitivity of the test is 66.6%

The specificity of the test is 93.2%

If at the clinical endpoint patients from point c) turn out to be patients with COVID19, then the specificity of the test will be higher by these cases.

LIST OF TESTS CARRIED OUT WITH THE PCL COVID19 Ag Rapid FIA SET

No.	COVID-19 (SARS-COV-2) RNA met.RT-PCR test GeneFinder COVID19 Plus Real Amp kit ELITEInGenius device	PCL COVID19 Ag Rapid FIA, series COV052003N401, COV052003N402
1.	Not detected	POSITIVE
2.	Not detected	negative
3.	Not detected	negative
4.	Not detected	negative
5.	Not detected	POSITIVE
6.	DETECTED GENE RdRP, GENE N, GENE E / CT 25	POSITIVE
7.	Not detected	negative
8.	Not detected	negative
9.	Not detected	POSITIVE
10.	Not detected	negative
11.	DETECTED GENE N / CT 38,8	negative
12.	Not detected	negative
13.	Not detected	negative
14.	Not detected	negative
15.	Not detected	negative
16.	Not detected	negative
17.	Not detected	negative
18.	Not detected	POSITIVE
19.	Not detected	negative
20.	Not detected	negative
21.	Not detected	negative
22.	Not detected	negative
23.	Not detected	negative
24.	Not detected	negative
25.	DETECTED GENE RdRP, GENE N, GENE E / CT 28	POSITIVE
26.	DETECTED GENE RdRP, GENE N, GENE E / CT 23	POSITIVE
27.	DETECTED GENE RdRP, GENE N, GENE E / CT 26	POSITIVE
28.	DETECTED GENE N / CT 36	negative
29.	DETECTED GENE RdRP, GENE N, GENE E / CT 30	negative
30.	DETECTED GENE RdRP, GENE N, GENE E / CT 35	negative
31.	DETECTED GENE N / CT 31	negative
32.	DETECTED GENE RdRP, GENE N, GENE E / CT 29	POSITIVE
33.	DETECTED GENE RdRP, GENE N, GENE E / CT 26	POSITIVE
34.	DETECTED GENE RdRP, GENE N, GENE E / CT 28	POSITIVE
35.	Not detcted	negative
36.	DETECTED GENE RdRP, GENE N, GENE E / CT 25	POSITIVE
37.	DETECTED GENE RdRP, GENE N, GENE E / CT 26	POSITIVE
38.	DETECTED GENE RdRP, GENE N, GENE E / CT 24	POSITIVE

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39.	DETECTED GENE RdRP, GENE N, GENE E / CT 28	POSITIVE
40.	DETECTED GENE RdRP, GENE N, GENE E / CT 33	negative
41.	Not detected	negative
42.	Not detected	negative
43.	Not detected	negative
44.	Not detected	negative
45.	Not detected	negative
46.	Not detected	negative
47.	Not detected	negative
48.	Not detected	negative
49.	Not detected	negative
50.	Not detected	negative
51.	Not detected	negative
52.	Not detected	negative
53.	Not detected	negative
54.	Not detected	negative
55.	Not detected	negative
56.	Not detected	negative
57.	Not detected	negative
58.	Not detected	negative
59.	Not detected	negative
60.	Not detected	negative
61.	Not detected	negative
62.	Not detected	POSITIVE
63.	Not detected	negative
64.	Not detected	negative
65.	Not detected	negative
66.	Not detected	negative
67.	Not detected	negative
68.	Not detected	negative
69.	Not detected	negative
70.	Not detected	negative
71.	Not detected	negative
72.	Not detected	negative
73.	Not detected	negative
74.	Not detected	negative
75.	Not detected	negative
76.	Not detected	negative
77.	Not detected	negative
78.	Not detected	negative
79.	Not detected	negative
80.	Not detected	negative

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81.	Not detected	negative
82.	Not detected	negative
83.	Not detected	negative
84.	Not detected	negative
85.	Not detected	negative
86.	Not detected	negative
87.	Not detected	POSITIVE
88.	Not detected	negative
89.	Not detected	negative
90.	Not detected	negative
91.	Not detected	negative
92.	Not detected	negative
93.	Not detected	negative
94.	Not detected	negative
95.	Not detected	negative
96.	Not detected	negative
97.	DOUBTFUL GENE N / CT 29	POSITIVE
98.	Not detected	negative
99.	Not detected	faulty test / sample reading error
100.	Not detected	faulty test / sample reading error

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Załącznik 3

Regional Infectious Disease Hospital in Warsaw

Central Analytical Laboratory /CAL/

TEST REPORT - PCL TEST COVID19 Ag Rapid FIA

Test series number: COV05-2003N405 + one box of the COV05-2003N401 series (with screw-on tubes with extraction buffer)

266 antigen (Ag) determinations were carried out. All samples were determined in parallel with an RT-PCR test.

COVID-19 was detected by RT-PCR in 40 samples. 16 positive Ag results (convergent with RT-PCR results) were obtained.

In addition, 5 false positive results were obtained. In 3 cases they concerned HIV + patients.

Based on these data, the following were calculated:

Test sensitivity - 40%

Test specificity - 98%

Ct values of RT - PCR test, at which positive results of Ag Rapid FIA test were obtained, ranged between 17 - 28, false negative results were obtained at Ct values in the range of 26 - 39.

Technical Notes:

1. Extraction buffer tubes plugged with pressed plugs are not always tight. The test material leaks during transport. Screw-on tubes are better.

2. The instruction in the test description recommends applying 1-2 drops of the sample. There were two drops applied, and yet it happened that the reader did not detect the sample and instead of the result the device indicated "no sample or insufficient sample volume".

3. In the last few determinations, the results were read according to the instructions after 10 minutes and then once again, after additional 10 minutes, prolonging the incubation. This did not change the result (applies to negative results).

CONCLUSIONS

1. At 40% sensitivity the test is not suitable for routine diagnostics.

2. One pack of one series is not enough for evaluation.

Hanna Czeszko – Paprocka

Head of CAL

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Załącznik 4

Last week [4-8 May 2020], at the Hospital Emergency Department, we performed rapid antigen tests (PCL COVID19 Rapid FIA kit in 265) patients admitted to University of Bialystok Clinical Hospital, who were at the same time swabbed for genetic (PCR) testing. We obtained 265 negative results in antigenic tests, which were confirmed by negative genetic tests results. Thus, it can at least be assumed that rapid antigen tests do not give false positive results.

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Załącznik 5

Bytom, 18/05/2020
Results of PCL Covid 19 Ag Rapid test evaluation
(LOT no. COV05-2003N402, expiry date 2022.03.02)
Clinical Department of Infectious Diseases and Hepatology in Bytom,
Medical University of Silesia in Katowice

Table. Clinical, biochemical and virological characteristics of the examined patients

Patient	Sex, age	Covid-19 stadium	Hospitalized from	Saturation*	IL-6 / D-dimer*	PCR data	PCL data
HM	M, 67	ARDS, respiratory failure, referred to ICU, diabetes	10.05	72%	245 pg/ml 1673 ng/mL	06.05 positive	11.05 negative
RW	M, 55	Pneumonia, diabetes	23.04	91%	<1.5 pg/mL 484 ng/mL	23.04 positive 11.05 negative	11.05 negative
MH	M, 80	Pre-ARDS, hypertension	06.05	79-84%	35.7 pg/mL 2555 ng/mL	08.05 positive	11.05 negative
PM	M, 59	Pneumonia, fever	27.04	86%	4.4 pg/mL 286 ng/mL	29.04 positive	11.05 negative
LJ	F, 60	Pneumonia, diarrhea	28.04	91%	75 pg/mL 3903 ng/mL	29.04 positive	11.05 negative
KK	M, 49	Pre-ARDS, respiratory failure, diabetes	11.05	70%	242 pg/mL 8245 ng/mL	12.05 positive	15.05 negative
MH* Second test of the same infected patient	M, 80	Pre-ARDS, hypertension	06.05	70%	IL-6 not performed D-dimer 2870 ng/mL	08.05 positive	15.05 negative

PE	F, 85	Pre-ARDS, respiratory failure, fever	10.05	80%	313 pg/mL 298	13.05 positive	15.05 negative
JH	F, 69	Pneumonia, contact with a SARS + person	08.05	88%	3.47pg/ml 1650 ng/ml	12.05 negative	15.05 negative
KW	M, 56	Pneumonia, son is SARS +	09.05	97%	49 pg/mL 422 ng/mL	12.05 negative	15.05 negative

*results closest to the PCL test date

In addition, 5 tests of medical personnel were performed, whose PCR and PCL results were consistently negative.

Qualitative determination of the presence of RNA virus SARS-CoV-2 was carried out by real-time polymerase chain reaction technique (Real Time multiplex PCR technique) preceded by reverse transcription. The MutaPLEX Coronavirus Real-Time-RT-PCR Kit, Immundiagnostik AG (IVD) test was used. Read: CFX96 Real Time System (Bio-Rad). Sensitivity for SARS-CoV-2: ≤500 RNA copies / ml.

Summary - in the conducted cohort there was not a single positive result for SARS-Cov-2 antigen.

This is puzzling because nasopharyngeal swabs were tested on patients with COVID-19 symptoms (in the phase of pneumonia or pre-ARDS / ARDS), and in some within 1-3 days after the onset of symptoms, i.e. during the highest viral load /viremia/ in the respiratory tract. Some patients were also tested at later stages of the disease (details in the Table above). The tests were conducted within a maximum of 15 minutes after swabbing, at room temperature according to the protocol. The lack of positive control is also puzzling, which is a good laboratory practice – this lack makes it impossible to verify the efficiency of the device, because the self-test function carried out before each round of determinations indicates the absence of defects only.

The presented results do not encourage further testing of the device.

Yours sincerely,

Jerzy Jaroszewicz, MD, PhD

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Załącznik 6

Centre for Diagnostics and Combating Biological Hazards in Puławy Poland

The PCL COVID19 Ag Rapid FIA diagnostic kit is an in vitro diagnostic medical device using a fluorescent immunoassay (Sandwich FIA) as a test method. The kit is used for the qualitative detection of SARS-CoV-2 antigen in clinical samples taken from the oropharynx / nasopharynx and sputum samples. The manufacturer limits the detection limit of the detected viral antigen to 0.1 ng / ml.

After preliminary checks carried out at the Centre for Diagnostics and Combating Biological Hazards the following can be stated:

- the diagnostic kit contains all necessary test accessories: swabs / spatulas for sample collection (Sample Swab), reagents (Sample Diluent) in divided tubes (1 buffer for 1 test sample) with a suitable stopper necessary for applying the sample to the test window (Filter Cap) , Test Cards and the Identification Card assigned to each reagent packaging (ID Chip Card). The set is complete and does not require the operator to use additional accessories.
- diagnostic procedure - simple and easy to use (strictly following the manufacturer's instructions); however, since it requires working with potentially infectious material, it is recommended that the kit is used by qualified medical personnel, in particular as it requires collecting the sample from the patient correctly.
- PCLOK EZ test reader / analyser - a compact device for reading, analysing and interpreting immunological tests in standard mode (duration - 10 minutes) and fast mode (duration - several seconds). Intuitive and operator friendly software (detailed manufacturer's instructions included). The result is displayed on the PCLOK EZ screen based on the fluorescent signal of the control line and test line, it is archived (testing control and monitoring) and can be printed directly from the device. An important element of the system is quality control (QC test) in the form of an additional test card (Card Calibration), which ensures the correct reading of the analysed results.

Summary:

PCL COVID19 Ag Rapid FIA diagnostic kit	
Advantages	Limitations
• diagnostic kit - small, compact, mobile device - can be used in any, even small, laboratory space. • et equipped with all possible research accessories, which means that it does not require the use of additional equipment • analyser equipped with intuitive, operator - friendly research software. • a quick diagnostic method, recommended for screening in addition to other diagnostic methods and initial screening. • low cost of the test. • test analyser / reader - a device simple and intuitive to use, supporting the analysis process and limiting the reading error due to greater sensitivity than the visual reading of the operator.	• power supply only from the network, no battery power available which would increase the mobility of the set. I think there is a battery slot - for verification • despite the simplicity of diagnostics, the kit should be used by qualified medical staff and in a designated place (laboratory, emergency ward, hospital ward, etc.), not intended for "home use". • the analysis is only qualitative - no information about the amount of antigen in the sample. • detection limit - the level of detection below which the antigen is not detected (possibility of false negative results). • Other restrictions specific to the used immunoassay method like: matrix effect, overflow effect, cross-reactions. • Read option - Quick test is not recommended (high read error - false negative results)

Results of research conducted:

On 5-7.05.2020, the analytical and diagnostic team checked the PCL COVID19 Ag Rapid FIA device and dedicated tests on patients in the isolation room who had previously confirmed SARS-CoV-2 infection by RT-qPCR method. In addition, parallel studies were conducted using rapid cassette tests in which capillary blood was the diagnostic material. After collecting nasopharyngeal swabs (according to the manufacturer's instructions), approximately 20 samples were analysed using the PCL COVID19 Ag Rapid FIA device. The obtained results did not show the presence of the virus in any of the tested samples. At the same time, comparative tests performed using rapid cassette tests confirmed the presence of antibodies against SARS-CoV-2 in the 4 samples tested. It should be noted that all patients in the isolation room were previously examined for the presence of SARS-CoV-2 virus using the RT-qPCR method.

The next stage of testing the kit was conducting tests / validation of the kit in laboratory conditions using positive samples from previously diagnosed patients (based on conducted and confirmed genetic tests). The starting material for the study were throat swab samples taken from hospitalized patients – these samples were tested for SARS-CoV-2 using genetic methods. Selected

positive samples were tested using the PCL COVID19 Ag Rapid FIA diagnostic kit. The conclusions of this research experiment were as follows: positive results with the use of the above set were confirmed only in 50% of the examined cases using the standard reading mode, while the Quick test reading analysed the tested samples as negative.

To sum up, the obtained results of tests carried out on samples from patients with confirmed SARS-CoV-2 infection, staying in an isolation room and undergoing pharmacological therapy testify to a low level of virus detection using the tested method. The above may result from the potential impact of pharmacotherapy on the test result, however, according to the manufacturer's assurances no impact of medicinal products (including diphenhydramine, guaiacol glycerol ether, oxymetazoline, phenylephrine, amantadine, ribavirin, pseudoephedrine hydrochloride, ibuprofen, oseltamivir) on the test result was observed. There seems to be a need for further research that would allow verification of the manufacturer's assurances in this area. In addition, testing of the kit using confirmed positive samples for SARS-CoV-2 should be carried out for a larger pool of samples and the final analysis and interpretation of the result should include correlations with the type of clinical specimen collected, the stage of infection at which the sample was taken, and in relation to the obtained test results genetic.

Remarks:

1. Results obtained using the PCL COVID19 Ag Rapid FIA device should not be considered final and should not be the sole basis for treatment or patient management. Each test based on the above method should be confirmed by other diagnostic tests based on a different test method, e.g. RT-qPCR, ELISA.
    - In the early stages of infection, low levels of antigen expression can produce false negative results. Each test has a limited sensitivity (detection limit), the infectious dose may be lower than the test sensitivity. Despite the negative result, there may be some agent in the sample that could cause disease.
2. Due to the limitations of the method, the negative results obtained cannot constitute grounds for completely excluding the possibility of infection. The tested device only enables the qualitative identification of SARS-CoV-2 antigen in human oropharynx / nasopharynx and sputum samples and does not allow quantitative determination of the amount of antigen in the sample.
3. Other diagnostically relevant limitations of the method are:

    matrix effect - when in the test sample, apart from the proper agent, there may also be another agent preventing the binding of the antigen to the antibody. A false positive result is obtained when the agent present in the sample (not the antigen tested) binds non-specifically to the antibody.

    possible cross-reactions - this effect may cause false positive results in the case where two closely related species share a common antigenic epitope, the antibody may in this case

interact with both related antigens (false positive). The manufacturer ensures no cross-reactions, but only for pathogens selected by him.

overfill effect - can occur when too much antigen is introduced into the test. The amount of antigen exceeds the amount of colloidal gold particles, the excess of antigen travels along the test faster than the heavier bound antigens thus filling the binding sites of the proper antibody-antigen complex - the result may be a false negative result.

* all the above limitations should be taken into account when interpreting the test results

RECOMMENDATIONS:

1. After the tests and comparative analyses of the effectiveness of the COVID19 Ag Rapid FIA PCL device presented for assessment together with dedicated tests, they should be considered of little use in the diagnosis of COVID 19, due to the significantly limited reliability of the results obtained in relation to the recommended RT-qPCR method , which is the "gold standard" for diagnosing SARS-CoV-2 infection[8],[9].
2. Despite the undoubted advantages of the device, which is the speed of analysis and its low cost, the device presented for testing should be considered of little use in medical diagnostic laboratories.
3. The small size and high mobility of the device and the speed of analysis indicate its usefulness in rapid clinical diagnostics (e.g. in intensive care units, emergency rooms) and in other medical care facilities, however, the insufficient percentage of reliable, repeatable results does not allow for giving a positive recommendation at this moment.

[8] Recommendations in COVID-19. Polish diagnostic and therapeutic and organizational recommendations for the care of persons infected or exposed to SARS-CoV-2 infection. Agency for Health Technology Assessment and Tariffication. Version 1.0 of 23.04.2020

[9] NIZP-PZH recommendations in the field of SARS-CoV-2molecular diagnostics. National Institute of Hygiene, version of 24.04.2020.