Performance Evaluation of IVD Medical Device

1. Product identification

Name: **Bi-CoV™ set**

Lot number: 01-2020

Manufacturer: Bioinova, s.r.o., Vídeňská 1083, 142 00 Prague 4, Czech Republic

Bi-CoV™ set is a collection kit used for noninvasive sampling of biological material from the oral cavity or nasopharynx by soaking the fluid into an inserted swab, inserting it into a plastic tube containing a collection (transport) solution and for subsequent transport of the sample to the laboratory for PCR analysis.
The solution in the kit ensures a gradual decomposition of the viral particles (decrease in virulence by 68.4% after 1 h and by 96.6% after 24 h), release of nucleic acids (RNA) into the solution and their protection against degradation. The sample is ready for PCR analysis without the need to perform an RNA isolation in the diagnostic laboratory.

2. Performance evaluation was done by

Bioinova, s.r.o., Vídeňská 1083, 142 00 Prague 4, Czech Republic

Evaluator:
M.D. Peter Bauer, Ph.D.
Bioinova, s.r.o., Vídeňská 1083, 142 00 Prague 4, Czech Republic
e-mail: peter.bauer@bioinova.cz

3. Evaluation plan

Patients who arrived at the collection point of AGEL Hospital in Nový Jičín (Nemocnice AGEL Nový Jičín a.s.) for a standard SARS-CoV-2 PCR test were given the opportunity to participate in the performance evaluation. Patients were informed about the purpose of the evaluation and that additional samples would be required. Those who agreed signed the informed consent form (see Annex). The design of the performance evaluation was approved by the local ethics committee. Each consented patient underwent:

A. Nasopharyngeal swab using a standard PCR collection kit

B. Nasopharyngeal swab using Bi-CoV™ set (see Instructions for Use)

C. Collection of saliva from the oral cavity using Bi-CoV™ set (see Instructions for Use)

All samples were analyzed by PCR. Samples B and C were analyzed without prior RNA isolation.

The performance evaluation shall be repeated if the composition of the sampling solution in Bi-CoV™ set changes.

4. Material

For sample collection:

A. standard PCR collection kit

Viral Transport Tube by Jiangsu Rongye Technology Co., Ltd., lot no. 20200918

B. Bi-CoV™ set

C. Bi-CoV™ set

5. Methods

5.1. Sample collection

A. Standard procedure of the hospital collection point (Nemocnice Agel Nový Jičín a.s.)

B. Instructions for Use of Bi-CoV™ set (Bioinova, s.r.o.)

C. Instructions for Use of Bi-CoV™ set (Bioinova, s.r.o.)

5.2. RNA isolation

according to BI-ZP-SOP-01 using EliGene Viral RNA / DNA FAST Isolation Kit (Elisabeth Pharmacon, cat. no. 409100)

5.3. PCR detection

according to BI-ZP-SOP-02 using GeneProof SARS-CoV-2 PCR Kit (GeneProof, cat. no. COV2 / GP / 100)

6. Analytical procedure

The collected samples were analyzed by PCR. The result of sample A, which was taken into a standard PCR collecting kit and analyzed after previous RNA isolation, was used as the reference procedure. Samples B and C were analyzed by PCR without prior RNA isolation. The results of all three analyzes should be identical for each sample. Because three samples had to be taken from each patient, it may not have been possible to collect these samples in exactly the same way.

Therefore, in case of discrepancy between the result of a reference sample A and related samples B or C, the PCR analysis of the samples B or C was repeated with previous RNA isolation. If sample A was positive, but samples B and C remained negative even after isolation, the discrepancy of the results was caused by incorrect sampling and not by malfunction of the collection solution in Bi-CoV™ set.

7. Evaluation

Samples were collected between 25th and 26th November 2020. Three samples were taken from each patient:

A. Nasopharyngeal swab using a standard PCR collection kit

B. Nasopharyngeal swab using Bi-CoV™ set (see Instructions for Use)

C. Saliva sample from the oral cavity using Bi-CoV™ set (see Instructions for Use)

GeneProof SARS-CoV-2 PCR Kit, which is normally used in Bioinova laboratoy for the detection of SARS-CoV-2 by RT-qPCR (reverse transcription quantitative polymerase chain reaction), was used to analyze the samples. The GeneProof kit detects specific sequences of the viral RdRp, E and N genes in a single reaction, providing high sensitivity of a virus detection. The detection kit uses a "hot start" polymerase that minimizes non-specific reactions and ensures maximum sensitivity. When comparing samples A, B and C, the specific sequence of the viral genes RdRp/E was evaluated as authoritative and thus decisive in the detection of SARS-CoV-2. This sequence was detected in the FAM channel (green channel). The signal of N gene (red channel) may not be detectable in patients with a lower SARS-CoV-2 viral load (higher C_T in the FAM channel).

7.1. Sensitivity

The evaluation was performed on a total of 113 samples that were collected.

	A	B	C
POSITIVE SAMPLES	27	38	37
NEGATIVE SAMPLES	86	75	76
TOTAL		113	

Table 1: Overview of the number of positive and negative samples taken by the standard PCR collection kit (A) and Bi-CoV™ set (B, C)

The analysis confirmed 100% agreement between the positive samples taken by the standard collection kit (A) and by Bi-CoV™ set (B, C). In addition to confirming the results of the standard collection kit, Bi-CoV™ set detected the presence of SARS-CoV-2 virus in additional 11 patients.

In 7 of these patients, the presence of SARS-Cov-2 was detected in in both nasopharyngeal swabs (B) and oral swabs (C), 3 patients had positive nasopharyngeal swabs only (B) and 1 patient had positive oral swab only (C). The presence of the virus in all positive B and C samples was further confirmed after a subsequent isolation, so there was no false positivity. In the case of B and C samples whose results did not match, the presence of the virus in negative samples was not proven even after a previous isolation. This means that the discrepancy in the results was caused by the sampling method and not by the malfunction of Bi-CoV defect ™ set.

	AGREEMENT	NOTES
STANDARD KIT VS BI-COV™ SET / NASOPHARYNX	100%	Bi-CoV ™ set (nasopharynx) detected additional 11 positive samples
STANDARD KIT VS BI-COV™ SET / ORAL CAVITY	100%	Bi-CoV ™ set (oral cavity) detected additional 8 positive samples
BI-COV™ SET / NASOPHARYNX VS ORAL CAVITY	92%	3x nasopharynx only; 1x oral cavity only; all these samples were negative when collected into the standard collection kit

Table 2: Sensitivity

The test results were determined based on the shape of the curve and the C_T value. The sample was considered positive if C_T<35 (see Annex 1). The average C_T values of positive samples are in Annex 2.

7.2. Specificity and reproducibility

Furthermore, the results were compared on 2 other detection kits with 100% agreement (see Annex 3).

Kits used for comparison:

1) gb SARS-CoV-2 Multiplex (GENERI BIOTECH, Cat. No. 3231-200)

2) ViroQ SARS-CoV-2 (BAG Diagnostics, Cat. No. 728250)

7.3. Sample stability in Bi-CoV™ set

The stability of the sample in Bi-CoV™ set was verified in selected positive samples after 48 hours (see Annex 4) and after 72 hours (see Annex 5). The analysis was performed after storage in a refrigerator. There was 100% agreement in the results at both time points (48 and 72 hours).

7.4. Start and end date of the evaluation

2nd November 2020 - 4th December 2020

8. Binding opinion of the evaluator

Due to the fact that it is not necessary to perform RNA isolation, sampling into Bi-Cov™ set collection kit is especially advantageous for laboratories that do not have automatic isolators. This method saves time and cost of the diagnostic laboratories and increases the testing capacity for the presence of SARS-CoV-2 virus. The inactivation ability of the solution increases safety of sample processing by personnel. The collection kit is suitable for the collection of material from both the nasopharynx and the oral cavity (including saliva).

The evaluated collection kit , Bi-Cov™ set, has at least comparable efficiency as commonly used collection kits.

In Prague on 4th December 2020

Evaluator: MUDr. Peter Bauer, Ph.D.

Digitálně podepsal MUDr. Peter Bauer, Ph.D.
Datum: 2021.03.22 13:11:52 +01'00'

Peter Bauer, M.D., Ph.D.
Bioinova, s.r.o.

Annex 1: Overall results of the performance evaluation

Sample Nr.	A) standard collection with RNA isolation	B) nasopharyngeal swab in Bi-CoV™ set	C) saliva in Bi-CoV™ set
1	negative	negative	negative
2	negative	negative	negative
3	negative	negative	negative
4	negative	negative	negative
5	negative	negative	negative
6	negative	negative	negative
7	positive	positive	positive
8	negative	positive	negative
9	positive	positive	positive
10	negative	negative	negative
11	negative	positive	positive
12	negative	negative	negative
13	negative	positive	positive
14	positive	positive	positive
15	negative	positive	positive
16	negative	positive	positive
17	negative	negative	negative
18	negative	negative	negative
19	positive	positive	positive
20	negative	negative	negative
21	negative	negative	negative
22	negative	negative	negative
23	negative	negative	negative
24	negative	negative	negative
25	negative	negative	negative
26	negative	negative	negative
27	negative	negative	negative
28	negative	negative	negative
29	negative	negative	negative
30	negative	negative	negative
31	negative	negative	negative
32	negative	negative	negative
33	negative	negative	negative
34	positive	positive	positive
35	negative	negative	negative
36	negative	negative	negative
37	negative	negative	negative
38	negative	negative	negative
39	positive	positive	positive

Sample Nr.	A) standard collection with RNA isolation	B) nasopharyngel swab in Bi-CoV™ set	C) saliva in Bi-CoV™ set
40	positive	positive	positive
41	negative	negative	negative
42	negative	negative	negative
43	negative	positive	positive
44	positive	positive	positive
45	negative	negative	negative
46	negative	negative	negative
47	negative	negative	negative
48	negative	negative	negative
49	positive	positive	positive
50	negative	negative	negative
51	negative	negative	negative
52	positive	positive	positive
53	negative	positive	positive
54	negative	negative	negative
55	negative	negative	negative
56	positive	positive	positive
57	negative	negative	negative
58	negative	negative	negative
59	positive	positive	positive
60	negative	negative	negative
61	negative	negative	negative
62	negative	negative	negative
63	positive	positive	positive
64	positive	positive	positive
65	negative	negative	negative
66	negative	negative	negative
67	positive	positive	positive
68	negative	negative	negative
69	positive	positive	positive
70	positive	positive	positive
71	positive	positive	positive
72	negative	negative	negative
73	positive	positive	positive
74	positive	positive	positive
75	negative	negative	negative
76	positive	positive	positive
77	negative	negative	negative
78	negative	negative	negative
79	negative	negative	negative

Sample Nr.	A) standard collection with RNA isolation	B) nasopharyngel swab in Bi-CoV™ set	C) saliva in Bi-CoV™ set
80	negative	negative	negative
81	positive	positive	positive
82	negative	positive	positive
83	negative	negative	negative
84	negative	negative	negative
85	positive	positive	positive
86	negative	negative	negative
87	negative	negative	negative
88	negative	negative	negative
89	negative	negative	negative
90	negative	negative	negative
91	negative	negative	negative
92	negative	positive	negative
93	positive	positive	positive
94	negative	negative	positive
95	negative	negative	negative
96	negative	negative	negative
97	negative	negative	negative
98	negative	positive	positive
99	negative	negative	negative
100	negative	negative	negative
101	negative	negative	negative
102	negative	negative	negative
103	negative	negative	negative
104	negative	negative	negative
105	negative	negative	negative
106	positive	positive	positive
107	positive	positive	positive
108	negative	negative	negative
109	negative	positive	negative
110	negative	negative	negative
111	negative	negative	negative
112	positive	positive	positive
113	negative	negative	negative

Annex 2: Average C_T values of positive samples

Sample Nr.	A) standard collection with RNA isolation	B) nasopharyngel swab in Bi-CoV™ set	C) saliva in Bi-CoV™ set
7	26.89	18.31	18.39
8	negative	23.55	N
9	33.89	26.52	28.5
11	negative	26.69	32.07
13	negative	24.61	25.33
14	31.59	22.19	28.69
15	negative	26.33	29.84
16	Negative	24.17	29.32
19	28.79	25.89	31.89
34	31.99	27.73	29.28
39	30.86	21.24	32.64
40	32.66	26.71	31.92
43	negative	21.98	30.44
44	30.77	27.93	32.63
49	33.97	29.26	31.91
52	28.41	20.09	29.84
53	negative	28.26	27.10
56	30.62	20.90	30.54
59	26.74	19.56	27.98
63	31.17	22.28	22.85
64	34.08	24.38	32.21
67	31.06	27.14	32.47
69	27.24	23.13	32.99
70	23.00	18.29	33.00
71	30.58	26.04	28.97
73	23.10	23.28	31.55
74	25.24	19.69	26.88
76	24.19	20.55	28.39
81	26.34	20.69	32.36
82	negative	29.56	29.02
85	32.03	30.02	28.625
92	negative	27.01	negative
93	26.72	19.63	26.92
94	negative	negative	26.54
98	negative	28.96	30.59
105	negative	negative	negative
106	25.61	18.03	25.86
107	26.58	18.18	20.51
109	negative	24.91	negative
112	20.59	16.54	24.59

Annex 3: Comparison of different detection kits using selected positive samples (the comparison is based on C_T values)

Sample Nr.	Gene Proof		Generi Biotech		ViroQ	
	nasopharyngeal swab	saliva	nasopharyngeal swab	saliva	nasopharyngeal swab	saliva
7	14.78	15.13	25.55	24.97	17.71	17.81
13	22.04	20.78	28.74	30.34	25.86	28.62
14	18.95	27.34	27.92	28.79	23.57	31.02
15	24.13	28.27	29.42	28.56	26.97	32.68
19	24.64	35.05	29.05	28.36	27.14	35.47
34	26.35	28.12	29.18	29.49	28.93	31.06
43	18.01	29.72	27.81	29.45	23.5	33.97
44	26.24	34.1	29.46	29.14	29.99	34.44
52	16.56	29.59	26.89	29.62	20.44	31.14
56	17.09	29.26	27.74	29.95	21.4	31.96
59	15.67	26.72	25.79	28.38	21.62	29.43
63	19.2	20.51	28.59	27.47	22.27	22.8
71	22.82	27.12	29.36	30.67	29	30.53
74	16.08	25.24	25.51	28.9	20.64	27.41
76	17.26	27.37	27.06	29.13	20.89	29.47
81	17.03	31.78	27.57	28.05	21.43	34.9
82	34.79	26.81	28.87	31.14	29.15	31.43
93	16.9	25.09	25.65	29	19.42	27.5
105	13.7	24.85	24.65	25.84	20.14	27.31
106	15.9	18.06	23.83	24.18	18.2	21.21
112	13.63	23.76	22.98	24.49	15.41	26.28

Annex 4: Stability of selected positive samples in Bi-CoV™ set after 48 h based on C_T values

Sample Nr.	Gene Proof		Generi Biotech		ViroQ	
	nasopharyngeal swab	saliva	nasopharyngeal swab	saliva	nasopharyngeal swab	saliva
7	14.78	15.13	25.55	24.97	17.71	17.81
13	22.04	20.78	28.74	30.34	25.86	28.62
14	18.95	27.34	27.92	28.79	23.57	31.02
15	24.13	28.27	29.42	28.56	26.97	32.68
19	24.64	35.05	29.05	28.36	27.14	35.47
34	26.35	28.12	29.18	29.49	28.93	31.06
43	18.01	29.72	27.81	29.45	23.5	33.97
44	26.24	34.1	29.46	29.14	29.99	34.44
52	16.56	29.59	26.89	29.62	20.44	31.14
56	17.09	29.26	27.74	29.95	21.4	31.96
59	15.67	26.72	25.79	28.38	21.62	29.43
63	19.2	20.51	28.59	27.47	22.27	22.8

Annex 5: Stability of selected positive samples in Bi-CoV™ set after 72 h based on C_T values

Sample Nr.	Gene Proof		Generi Biotech		ViroQ	
	nasopharyngeal swab	saliva	nasopharyngeal swab	saliva	nasopharyngeal swab	saliva
71	22.82	27.12	29.36	30.67	29	30.53
74	16.08	25.24	25.51	28.9	20.64	27.41
76	17.26	27.37	27.06	29.13	20.89	29.47
81	17.03	31.78	27.57	28.05	21.43	34.9
82	34.79	26.81	28.87	31.14	29.15	31.43
93	16.9	25.09	25.65	29	19.42	27.5
105	13.7	24.85	24.65	25.84	20.14	27.31
106	15.9	18.06	23.83	24.18	18.2	21.21
112	13.63	23.76	22.98	24.49	15.41	26.28