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Reg. N. CHE-247.162.504

NANOSENSOR SPECIFICITY & SENSITIVITY

Regarding the specificity and sensitivity of the developed nanosensor, the developed platform exhibits tunable sensitivity/specificity as evidenced within the ROC curve.

In this regard, the nanosensor showed sensitivity and specificity of 95% and 60% compared with data of RT-PCR as gold standard. This outcomes show that we have more positive outcomes compared with RT-PCR. Please note that RT-PCR can not detect about 30% to 60% of infected people which is mainly due to the method of extraction, quality of kits, sampling procedure, viral load of samples and quality of biological specimens. In this matter, this diagnostic kit the equipped with nanobodies can cover this hidden majority which is one of the main transferring chains of the COVID-19 within the world.

What is more, by using high quality ELISA kits developed by EUROIMMUN, we can see that the sensitivity is 100% and specificity is 85% at cutoff point of 0.2185 µA, while the specificity could also reach 100% by increasing the cutoff point to 0.3265 µA. This fact shows that by accurate determination of intensity and drawing the related ROC curve, the sensor could be used as a fast sensitive screening sensor by sensitivity of 100% or a confident diagnostic kit by specificity of 100 % upon declining the sensitivity and changing the considered cutoff point.

More evidence about this matter can be seen in following Table(s) and/or Figure(s).

Table 1. Outcome of evaluations in comparison with results of RT-PCR as gold standard.

Parameter	Formula	Percentage compared with RT-PCR (%)
Sensitivity	TP/(TP + FN)	95
Specificity	TN/(TN+FP)	60
Accuracy	(TP+TN)/(P+N)	81
False negative rate	FN/P	5
False positive rate	FP/N	40

* TP: true positive, FP: false positive, TN: true negative, FN: false negative, P: positive and N: negative; 100 samples were evaluated whereby using RT-PCR, 60 and 40 of them were found to be positive and negative, respectively; compared with RT-PCR, nanosensor showed following results: TP: 57, FP: 16, TN: 24 and FN: 3.

Table 2. Outcome of blind samples evaluation by the developed nanosensor in comparison with the ELISA kit with cutoff point of 0.2 µA; (P: positive, N: negative); (20 positive and 20 negative samples)

Parameter	Formula	Obtained Percentage (%)
Sensitivity	TP/TP+FN	100
Specificity	TN/TN+FP	85
Negative prediction value	TN/TN+FN	100
Positive prediction value	TP/TP+FP	86.95
False negative rate	FN/FN+TP	0
False positive rate	FP/FP+TN	15
False discovery rate	FP/FP+TP	13.04
Accuracy	(TP+TN)/P+N	92.5
False negative rate	FN/P	0
False positive rate	FP/N	15

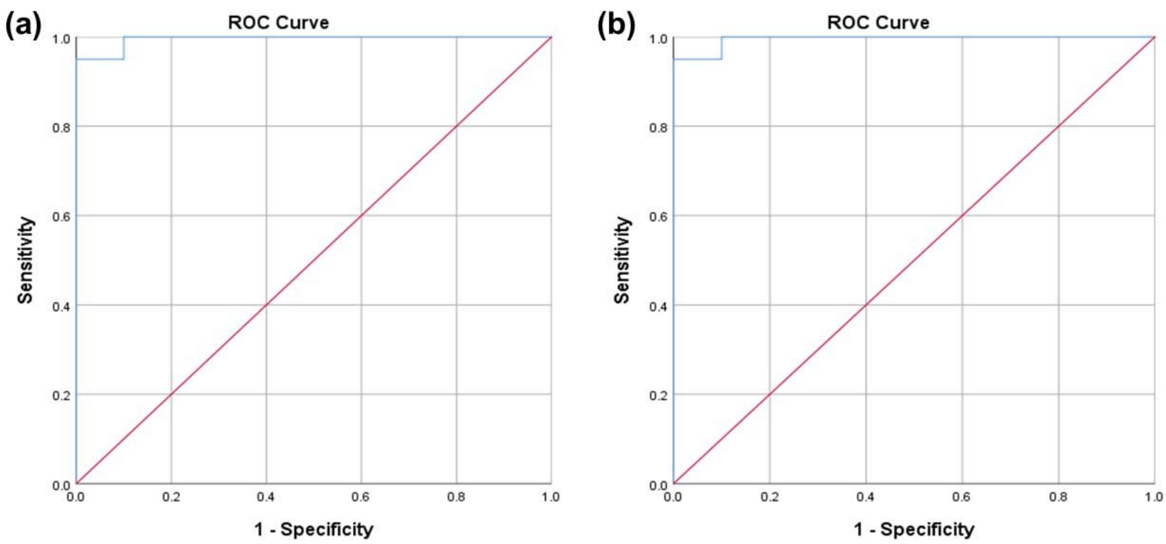

Figure 1. ROC curves related to (a) performance of the nansoensor toward detection of S1 proteins' antibody within blood samples and (b) correlation between the nanosensor and ELISA kits outcomes.

Table 3. Evaluation of area under curve of nanosensor's ROC curve (Figure 3 (a)); intensity of peaks based on µA was considered as test results variable(s).

Area	Standard error [a]	Asymptotic Significance [b]	Asymptotic 95 % Confident Interval	
			Lower bound	Upper bond
0.995	0.007	0.000	0.982	1.000

Note: a: under the nonparametric assumption and b: null hypothesis, true area=0.5

Table 4. Coordinates of the curve related to Figure 3 (a); intensity of peaks based on µA was considered as test results variable(s).

Positive if Greater Than or Equal To[a]	Sensitivity	1 - Specificity
-1.0000	1.000	1.000
.0760	1.000	.300
.1605	1.000	.250
.1750	1.000	.200
.2185	1.000	.150
.2585	1.000	.100
.2615	.950	.100
.2740	.950	.050
.3265	.950	.000
.3675	.900	.000
.4175	.850	.000
.4695	.800	.000
.5030	.750	.000
.5465	.700	.000
.5645	.650	.000
.5810	.600	.000
.6090	.550	.000
.6365	.500	.000
.6585	.450	.000
.6760	.400	.000
.6870	.350	.000
.6945	.300	.000
.6980	.250	.000
.7075	.200	.000
.7185	.150	.000
.7385	.100	.000
.8370	.050	.000
1.9180	.000	.000

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

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Table 5. Correlation between the obtained data from nanosensor and ELISA kits; ** correlation is significant at the 0.01 level (2-tailed).

		Peak Intensity (µA)	Ratio
Peak Intensity (µA)	Pearson Correlation	1	.717**
	Significance (2-tailed)	-	.000
	N	40	40
Ratio	Pearson Correlation	.717**	1
	Significance (2-tailed)	.000	
	N	40	40

Table 6. Correlation between obtained data from nanosensor and ELISA kit.

Variable Parameters	**Peak Intensity (µA)**	
	r	**p**
Ratio of ELISA result based on the optical density	0.717	0.001>

Note: Strong correlation, viz., p<0.001 and r=7.17, was observed between the obtained data from nanosensor and ELISA kit

Table 7. Evaluation of area under curve for correlation ROC curve between data of nanosensor and ELISA kit (Figure 3 (b)); intensity of peaks based on µA was considered as test results variable(s).

Area	**Standard error** [a]	**Asymptotic Significance** [b]	**Asymptotic 95 % Confident Interval**	
			Lower bound	**Upper bond**
0.995	0.007	0.000	0.982	1.000

Note: a: under the nonparametric assumption and b: null hypothesis, true area=0.5

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