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Clinical performance of the Elecsys electrochemiluminescent immunoassay for the detection of SARS-CoV-2 total antibodies

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To the Editor

In the context of COVID-19, a wide range of serology immunoassays with different SARS-CoV-2 antigen recognition and antibody specificity have been developed to complement RT-PCR assays *[1]*. Serological testing is useful for diagnosis and characterization of the course of the disease, identification of convalescent plasma donors, for epidemiology studies, lockdown exit programs and COVID-19 vaccine development *[2, 3]*. Due to the widespread dissemination of these new methods and the limited experience with these assays, it is crucial for laboratories to rigorously validate these methods before broad introduction into routine clinical practice. Independent validations are also needed to assure the assays are in line with expected analytical and clinical performance specifications *[1-4]*.

This study is the first to report the external validation of a new electrochemiluminescent immunoassay (ECLIA) test, the Elecsys anti-SARS-CoV-2 from Roche Diagnostics®. This test allows the detection of total antibodies (including IgG) specifically directed against SARS-CoV-2 nucleocapsid and is performed on the cobas® e801 module. The test result is given as a cut-off index (COI). According to the manufacturer, a result <1.0 is considered negative while a result ≥1.0 is considered positive *[5]*. The within-run and between-run imprecision (CV) on 5 patient pools (COI means of 0.081, 1.0, 8.7, 24, and 54) varied from 0.8% to 3.3%, and from 1.2% to 3.6%, respectively. Sample storage complied with the conditions listed in the package insert.

This retrospective study was conducted from May 6 to 12, 2020 at the clinical biology laboratory of the Clinique Saint-Luc Bouge (SLBO, Namur, Belgium). Serum samples (n=140) obtained from 97 patients with a confirmed RT-PCR SARS-CoV-2 diagnosis were used to determine the clinical sensitivity of the assay. RT-PCR on respiratory samples (nasopharyngeal swab samples) was performed on the LightCycler® 480 Instrument II using the LightMix® Modular SARS-CoV-2 E-gene set (Roche Diagnostics®). Serum samples were subdivided into

different categories based on the number of days after a positive RT-PCR test as follows: 0-6 days: 45 sera; 7-13 days: 35 sera; 14-20 days: 24 sera; 21-27 days: 15 sera; 28 days or more: 21 sera. Among the 60 samples collected 14 or more days after the RT-PCR positive detection, and using the manufacturer's cut-off, the Elecsys anti-SARS-CoV-2 immunoassay identified 55 true positive and 5 false negative samples. The diagnostic sensitivity was 91.7% (95%CI: 81.6-97.2%). Using the optimal cut-off provided by ROC curve analyses (i.e.>0.165) improved the performance of the test to give a sensitivity of 100% (95%CI: 94.0-100%) (**Figure 1**).

A sensitivity analysis was also performed considering the date of symptom onset. Among the 97 patients, data about time of symptom onset were available for 92 patients. The collected samples (n=129) were subdivided into different categories according to the number of days after the onset of symptoms as follows: 0-6 days: 22 sera; 7-13 days: 28 sera; 14-20 days: 26 sera; 21-27 days: 23 sera; 28 days or more: 30 sera. Among the 79 samples evaluated 14 or more days after the onset of symptoms, and using the manufacturer's cut-off, the Elecsys anti-SARS-CoV-2 assay identified 72 true positive and 7 false negative samples. The diagnostic sensitivity was 91.1% (95%CI: 82.6-96.4%). Using the ROC curve cut-off (i.e.>0.165) improved the performance of the tests with a sensitivity of 95.1% (95%CI: 88.0-98.7%). Analyses of serum samples obtained 28 days or more after symptom onset provided a sensitivity of 96.7% (95%CI: 82.8-99.9%) and 100% (95%CI: 88.9-100%) with the manufacturer and the optimized cut-off, respectively (**Figure 1**).

Considering samples obtained before 14 days (from RT-PCR + or symptoms onset), sensitivities were not sufficient to be reliable in clinical practice (**Figure 1**).

Non-SARS-CoV-2 sera (n=79) collected prior to the COVID-19 pandemic (between January 2019 and December 2019) with potential cross-reactions (cross-reactivity test group) were also analyzed. Samples in this group included positive antinuclear antibodies (n=5), anti-thyroglobulin antibody (n=1), anti-*Treponema pallidum* antibodies (n=2), antistreptolysin O

(n=1), anti-thyroid peroxidase antibodies (n=4), chikungunya antibody (n=1), direct Coombs (n=1), hepatitis B Ag (n=4), hepatitis C antibodies (n=7), hepatitis E antibodies (n=4), human immunodeficiency virus antibodies (n=2), IgA *Chlamydia pneumoniae* (n=1), IgG *Chlamydia trachomatis* (n=1), IgG *Coxiella burneti* (n=2), IgM *Borrelia* (n=1), IgM *Coxiella burneti* (n=1), IgM cytomegalovirus (n=5), IgM Ebstein Barr virus viral capsid (n=5), IgM *Mycoplasma pneumoniae* (n=6), IgM parvovirus B19 (n=7), IgM *Toxoplasma gondii* (n=5), influenza antibodies (n=6), RAI (search for irregular agglutinins) (n=2), and rheumatoid factor (n=5). The calculated specificity was 100% (95%CI: 95.44-100.0%). Using the ROC curve cut-off (i.e.>0.165) had no effect on the measured diagnostic specificity (**Figure 1**).

The optimal ROC cut-off showed excellent clinical performance 14 days or more following RT-PCR positivity or following the onset of COVID-19 symptoms. Additional studies are needed to further confirm the best cut-off. Expert societies are also urged to provide guidance on the best time after RT-PCR positivity or symptom onset to perform serological investigations, since this is an important determinant of the true positivity rate.

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Figure legend

Figure 1: Clinical performance of the Elecsys anti-SARS-CoV-2 assay subdivided by time since the RT-PCR positivity or since the onset of symptoms. Cross-reactivity refers to the cross-reactivity test group described in the text. * = unaffected by the cut-off used (≥1.0 or >0.165). The dotted lines indicate the manufacturer's cut-off (in black) and the optimized cut-off (in grey).

Figure 1

		Time from RT-PCR +					Time from symptom onset				
	n	0-6d 45	7-13d 35	14-20d 24	21-27d 15	≥28d 21	0-6d 22	7-13d 28	14-20d 26	21-27d 23	≥28d 30
COI ≥1.0	True positive	21	26	23	13	19	3	20	23	20	29
	False negative	24	9	1	2	2	19	8	3	3	1
	Sensitivity (%)	46.7	74.3	95.8	86.7	90.5	13.6	71.4	88.5	87.0	96.7
	95% CI	31.7-62.1	56.7-87.5	78.9-99.9	59.5-98.3	69.6-98.8	2.9-34.9	51.3-86.8	69.9-97.6	66.4-97.2	82.8-99.9
COI >0.165	True positive	22	30	24	15	21	5	24	24	21	30
	False negative	23	5	0	0	0	17	4	2	2	0
	Sensitivity (%)	48.9	85.7	100	100	100	22.7	85.7	92.1	91.3	100
	95%CI	33.7-64.2	69.7-95.2	85.8-100	78.2-100	83.9-100	7.8-45.4	67.3-96.0	74.9-99.1	72.0-98.9	88.4-100
	Specificity (%)			100					100		
	95%CI*			95.4-100					95.4-100