Mini Review

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Diagnostic performance of the fully automated Roche Elecsys SARS-CoV-2 antigen electrochemiluminescence immunoassay: a pooled analysis

Abstract

Objectives: Among the diagnostic tests that have recently become commercially available for diagnosing coronavirus disease 2019 (COVID-19), the fully-automated Roche Elecsys severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antigen electrochemiluminescence immunoassay (ECLIA) is one of the most widespread for its adaptability within a system of laboratory automation, rapidity and high-throughput. This article is aimed to provide the results of the first pooled analysis of its accuracy for diagnosing SARS-CoV-2 infections.

Content: We carried out an electronic search in Scopus and Medline, without language or date restrictions (i.e., up to January 18, 2022), to identify articles where the diagnostic performance of Roche Elecsys SARS-CoV-2 antigen ECLIA was compared with that of reference molecular diagnostic techniques.

Summary: Overall, 11 studies were identified, 10 of which (n=6,095 swabs) provided necessary data for inclusion in a pooled analysis. The pooled diagnostic sensitivity, specificity and area under the curve (AUC) in nasopharyngeal samples were 0.68 (95%CI, 0.66–0.70), 0.99 (95% CI, 0.99–0.99) and 0.958 (95%CI, 0.936–0.980), respectively. The cumulative observed agreement with reference molecular assays was 89.5% and the kappa statistic was 0.735 (95%CI, 0.716–0.754). The pooled diagnostic sensitivity in samples with high viral load (i.e., cycle threshold values <28–30) was 0.95 (95%CI, 0.92–0.97).

Outlook: The results of this pooled analysis confirm that the fully-automated Roche Elecsys SARS-CoV-2 antigen ECLIA has high diagnostic specificity and optimal diagnostic sensitivity for identifying nasopharyngeal samples with higher viral load, thus making it a reliable technique for mass screening and for supporting strategies based on shorten isolation and/or quarantine.

Keywords: antigen; COVID-19; diagnosis; immunoassay; SARS-CoV-2.

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Introduction

Although molecular testing remains the gold standard for diagnosing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections [1], it is now well established that coronavirus disease 2019 (COVID-19) population screening in public places (e.g., schools, stations, airports and so forth) or before large mass gatherings (i.e., sports events, concerts, public manifestations) by means of rapid and reliable tests would enable the prevention of a large number of SARS-CoV-2 infections, thus limiting viral circulation and spread [2]. This holds especially true for subjects freely circulating with asymptomatic SARS-CoV-2 infections, since the rate of infected persons without COVID-19 symptoms is high, typically ranging between 50 and 90% [3], and may be further increased after emergence and spread of the new Omicron (B.1.1529) variant [4]. Moreover, data suggests that SARS-CoV-2 is associated with a cumulative asymptomatic transmission rate as high as 25% [5].

The use of rapid antigen diagnostic tests is ideal for large population screenings as they can be performed on-site and do not require skilled personnel or dedicated instrumentation. Nevertheless, these assays still carry major drawbacks such as limited diagnostic accuracy, low throughput, arbitrary and qualitative interpretation of test results, as well as inability to directly transfer and store data within laboratory information systems or patient electronic health records for widespread, safe and long-term availability [6]. To this end, the possibility to deliver large volumes of samples to neighboring clinical laboratories, where they can be rapidly tested with fully-automated and high-throughput analytical techniques, represents a feasible solution to overcome the bottleneck of molecular testing caused by ongoing shortages of human and technical resources. As recently highlighted in a worldwide survey performed by the American Association of Clinical Chemistry (AACC), over 60% of worldwide respondent laboratories were unable to obtain supplies such as test kits and reagents for running all COVID-19 tests and nearly 90% reported shortage of testing personnel [7].

Among the existing diagnostic tests on the market (an updated list can be found in the FIND website) [8], the fully-automated Roche Elecsys SARS-CoV-2 antigen electrochemiluminescence immunoassay (ECLIA) is one of the most widely available, as this technique can be integrated within a system of laboratory automation (i.e., is available on analyzers of the COBAS series) and hence available in many worldwide laboratories. Briefly, this technique is based on monoclonal antibodies directed against the SARS-CoV-2 nucleocapsid (N) protein in double-antibody sandwich assay format. According to manufacturer's declarations [9], the sample volume is 30–50 μL, the turn-around time is around 18 min, sample are considered "reactive" when the cut-off index (COI) is ≥1, the limit of detection is as low as 22.5 Median Tissue Culture Infectious Dose (TCID50)/mL, whilst the total imprecision is reported between 1.7 and 5.8%. Additional information (e.g., pre-analytical indications, use of viral inactivation buffers and so forth) and biosafety requirements is available in the online package inset [9]. The aim of this study was to perform the first pooled analysis of the cumulative performance of this fully-automated technique for diagnosing SARS-CoV-2 infections, since understanding the cumulative performance of this assay will help informing and guiding the use in clinical and public health contexts.

Materials and methods

We carried out an electronic search in Scopus and Medline (PubMed interface) using the keywords "Roche" OR "Elecsys" AND "antigen" AND "SARS-CoV-2" or "COVID-19" within all search fields and without language or date restrictions (i.e., up to January 18, 2022), with the purpose of identifying articles where the diagnostic performance of the Roche Elecsys SARS-CoV-2 antigen fully-automated ECLIA was compared with a reference molecular diagnostic technique. Two authors (G.L. and B.M.H.) scrutinized title, abstract and full text (when available) of all results using the aforementioned search criteria, selecting those investigations in which the rates of true positive (TP), true negative (TN), false positive (FP) and false negative (FN) cases were provided and could be used for constructiong a 2 X 2 table. The references of all articles were also hand-searched for detecting other potentially eligible publications. The data from each eligible study was then included in a pooled analysis for estimating the cumulative diagnostic sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR) and accuracy (Summary Receiver Operating Characteristic Curve [SROC]; Agreement; Kappa statistics) with 95% confidence interval (95%CI). A random effects model was adopted, whilst heterogeneity was estimated with χ^2 test and I^2 statistic. Sub-analysis was performed in samples with high viral load. The statistical analysis was conducted using Meta-DiSc 1.4 (Unit of Clinical Biostatistics team of the Ramón y Cajal Hospital, Madrid, Spain) [10]. The study is in agreement with the Declaration of Helsinki and within the terms of local legislation.

Results

The electronic search according to the predefined criteria identified 55 publications after eliminating duplicates among the two scientific databases. Forty-four articles were excluded because they did not show data on Elecsys SARS-CoV-2 antigen ECLIA (n=37), did not contain clinical evaluation of the test (n=4), and were review articles (n=2) or correspondence (n=1). A final number of 11 studies (n=6,130 samples) could hence be included in this study [11–21], all of which except for one contained sufficient information for pooling the diagnostic performance (the study of Mak et al., n=35 samples, was excluded since no data on negative molecular tests were included) [16].

The main characteristics of the selected studies are summarized in Table 1. Three studies were conducted in Italy, two in Germany and one each of the remaining six investigations in Belgium, China, Cuba, Japan, Pakistan and Switzerland. In all studies except one, the diagnostic performance was assayed in nasopharyngeal swabs, whilst in the remaining study it was evaluated in both

Table 1: Summary of studies that investigated the cumulative diagnostic performance of the fully-automated Roche Elecsys SARS-CoV-2 antigen ECLIA.

Study	Country	Sample matrix	Sample size	Molecular assays (target genes)	Viral load
Audigé et al. [11]	Switzerland	Nasopharyngeal swabs	320	Roche Cobas SARS-CoV-2 IVD test (E)	14–40 Ct
Ben Abdelhanin et al. [12]	Belgium	Nasopharyngeal swabs	225	Seegene Allplex 2019-nCoV assay (E and N)	≤35 Ct
Hirotsu et al. [13]	Japan	Nasopharyngeal swabs	637	In-house assay derived from the Japanese National Institute of Infectious Diseases test (N)	Unavailable
Iqbal et al. [14]	Pakistan	Nasopharyngeal swabs	170	Roche TibMol Biol Real-Time PCR test (ORF1ab and RdRP)	≤35 Ct
Kolesova et al. [15]	Italy	Nasopharyngeal swabs	110	MutaPLEX SARS-COV-2 kit (E, S and RdRP)	14–40 Ct
Mak et al. [16]	China	Nasopharyngeal swabs	35	In-house assay (ORF1ab)	14–35 Ct
Montalvo Villalba et al. [17]	Cuba	Nasopharyngeal swabs	523	Sentinel STAT-NAT COVID-19 multiplex (RdRP and ORF1b)	16–38 Ct
Mueller et al. [18]	Italy	Nasopharyngeal swabs	403	Cepheid Xpert Xpress SARS-CoV-2 test (E and N)	19–40 Ct
Nörz et al. [19]	Germany	Nasopharyngeal swabs	3,139	Roche Cobas SARS-CoV-2 IVD test (E)	5.1×10^2–3.5×10^6 copies/mL
Osterman et al. [20]	Germany	Oro-nasopharyngeal swabs	408	Multiple assays – Seegene Allplex, Roche Cobas and Cepheid GeneXpert System (unspecified gene targets)	0.8×10^2–1.6×10^9 Geq/mL
Salvagno et al. [21]	Italy	Nasopharyngeal swabs	160	Altona RealStar SARS-CoV-2 RT-PCR Kit (E and S)	14–42 Ct

Ct, cycle threshold; ECLIA, electrochemiluminescence immunoassay.

nasopharyngeal and oropharyngeal swabs [20]. In one study, the diagnostic performance was also assayed in saliva [11]. The sample size ranged between 35 and 3,139. A sub-analysis of diagnostic sensitivity in samples with high viral load (i.e., Ct values <28–30) could be carried out in 6/11 of such articles (n=531 samples), since the others did not provide sufficient data stratified according to the viral load for enabling our calculation (Table 2).

The pooled cumulative diagnostic performance of Elecsys SARS-CoV-2 antigen ECLIA is summarized in Figure 1 (10 studies, n=6,095 swabs). The pooled diagnostic sensitivity, specificity and AUC were 0.68 (95%CI,

0.66–0.70; I^2, 96.5%), 0.99 (95%CI, 0.99–0.99; I^2, 91.3%) and 0.958 (95%CI, 0.936 to 0.980), respectively. The cumulative observed agreement was 89.5% and the kappa statistic was 0.735 (95%CI, 0.716–0.754). The pooled NLR and PLR were 0.32 (95%CI, 0.23–0.45 I^2, 96.8%) and 68.12 (95%CI, 19.06–243.44; I^2, 87.7%), respectively. The pooled diagnostic sensitivity in nasopharyngeal samples with high viral load (i.e., Ct values <28–30; 6 studies, 531 swabs) was 0.95 (95%CI, 0.92–0.97; I^2, 71.5%) (Figure 2). The diagnostic specificity and accuracy in high viral load nasopharyngeal samples could not be calculated since the samples were unavailable for this sub-analysis. In the only

Table 2: Summary of studies which investigated the diagnostic performance of the fully-automated Roche Elecsys SARS-CoV-2 antigen ECLIA in nasopharyngeal samples with high viral load (i.e., cycle threshold values <28–30).

Study	Country	Sample matrix	Sample size	Viral load
Audigé et al. [11]	Switzerland	Nasopharyngeal swabs	85	<28 Ct
Hirotsu et al. [13]	Japan	Nasopharyngeal swabs	77	<30 Ct
Kolesova et al. [15]	Italy	Nasopharyngeal swabs	63	<28 Ct
Mak et al. [16]	China	Nasopharyngeal swabs	30	<28 Ct
Nörz et al. [19]	Germany	Nasopharyngeal swabs	223	$>10^4$ copies/mL (<~30 Ct)
Salvagno et al. [21]	Italy	Nasopharyngeal swabs	53	<30 Ct

Ct, cycle threshold; ECLIA, electrochemiluminescence immunoassay.
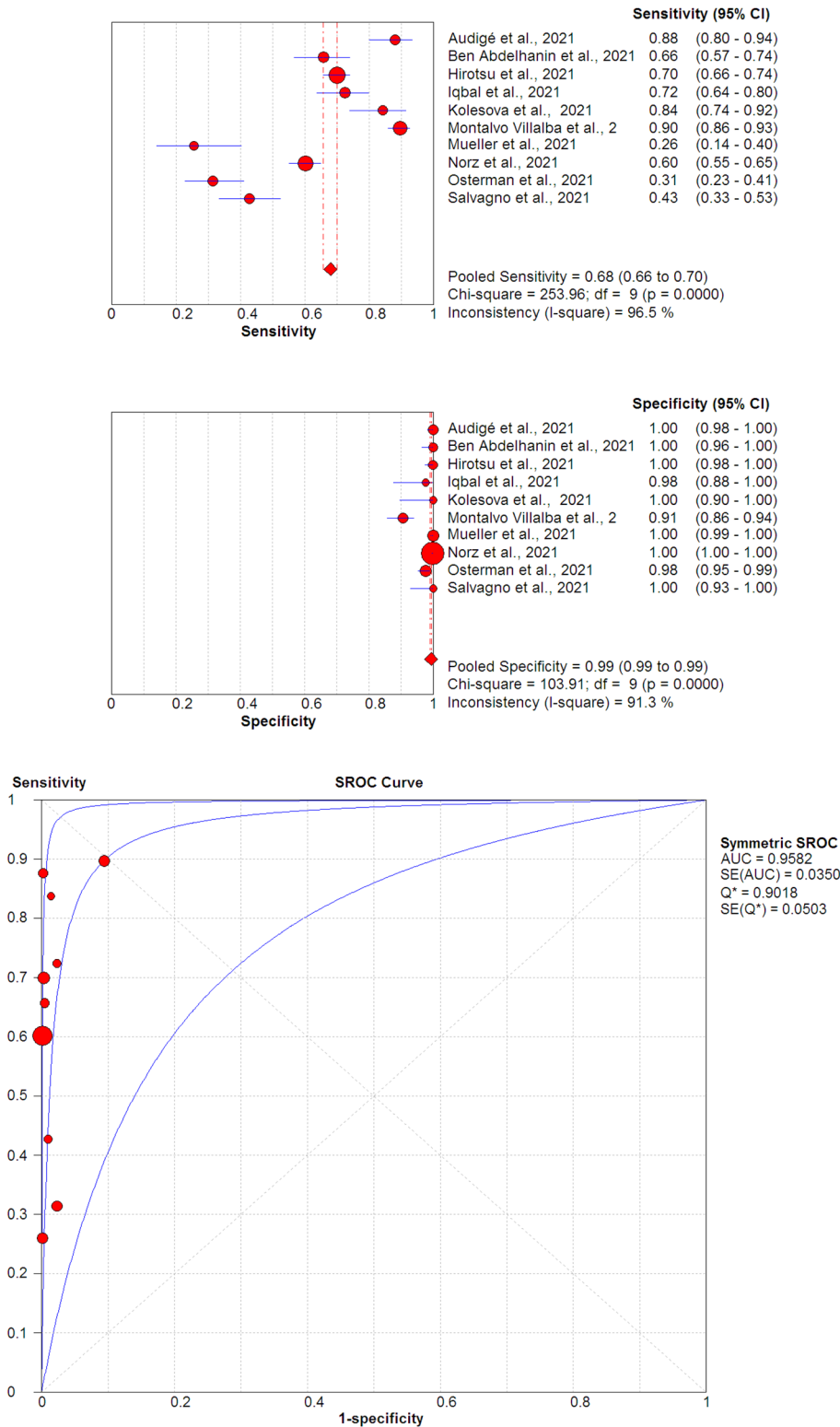
Figure 1: Cumulative diagnostic sensitivity, specificity and accuracy (Summary Receiver Operating Characteristic Curve [SROC]) with 95% confidence interval (95%CI) of the fully-automated Roche Elecsys SARS-CoV-2 antigen electro-chemiluminescence immunoassay (ECLIA) for diagnosing SARS-CoV-2 infection in nasopharyngeal samples.
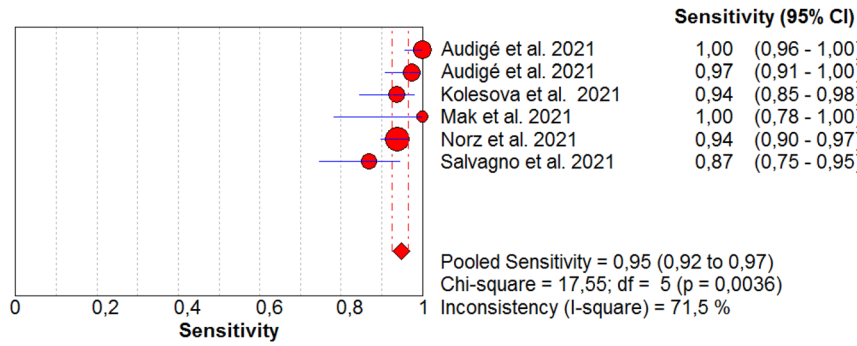
Figure 2: Diagnostic sensitivity of the fully-automated Roche Elecsys SARS-CoV-2 antigen electrochemiluminescence immunoassay (ECLIA) for diagnosing SARS-CoV-2 infection in nasopharyngeal samples with high viral load (i.e., Ct values <28–30).

Discussion

Two years after emergence of the worst human pandemic since the Spanish flu in 1918–1919, the global surge of SARS-CoV-2 positive cases does not seem to be declining, but instead is boosted by the spread of new and highly mutated variants [22]. These new linages appear more capable to escape both natural or vaccine-elicited immunity, thus they are responsible for a considerable number of re-infections and vaccine breakthroughs, ultimately imposing an unsustainable pressure on healthcare and diagnostic laboratories [23]. Although the molecular detection of SARS-CoV-2 RNA in upper respiratory tract specimens remains the reference technique for diagnosing COVID-19 [24], the increasing availability of high-throughput and fully-automated immunoassays aimed to rapidly and accurately identify SARS-CoV-2 antigens (namely the N protein) presents a promising solution for managing high volumes of diagnostic samples [25].

Despite some inherent limitations, such as variation in target genes, heterogeneous analytical sensitivity of reference molecular biology techniques and controversy on how thresholds for infectivity shall be defined [26], the results of this pooled analysis show that the fully-automated Elecsys SARS-CoV-2 antigen ECLIA has acceptable performance for diagnosing SARS-CoV-2 infection in nasopharyngeal samples, particularly in those with low Ct values, displaying 0.99 specificity and 0.96 AUC, respectively. The cumulative accordance and kappa statistic with reference molecular diagnostic techniques were ~90% and 0.735, respectively, thus reflecting substantial agreement [27]. The cumulative diagnostic sensitivity was instead found to be 0.68, thus implying a FN rate of around 32% in nasopharyngeal samples with a wide spectrum of viral loads. However, such suboptimal sensitivity increased to 95% in nasopharyngeal samples with Ct values <28–30. This implies that this technique could be reliably used to accurately identify subjects with high viral load, conventionally known as "super-carriers" and/or "super-spreaders", who may be responsible for a large number of infections and COVID-19-related hospitalizations [28, 29].

It is also noteworthy that the remarkably high (virtually perfect) diagnostic specificity that characterizes the Elecsys SARS-CoV-2 antigen ECLIA compared to reference molecular diagnostic techniques would enable the use of this accurate and reliable tool for social purposes, whereby suspension of quarantine and/or isolation after an acute SARS-CoV-2 infection mandatorily requires a negative SARS-CoV-2 test result in many countries [30]. Notably, detection of viral shedding with molecular biology techniques not always reflects infectivity, since positivity may be caused by detection of non-viable viral particles, free nucleic acids or RNA contained in debris-like tissue and degenerated cells, all conditions associated with a low/absent infective risk [31, 32]. To this end, this test (and probably other fully-automated immunoassays) could replace molecular detection of SARS-CoV-2 RNA to define whether a subject is still strongly colonized and highly contagious, thus legitimizing the adoption of shortened quarantine and/or isolation periods, provided that a reliable and validated SARS-CoV-2 tests was negative and that traditional physical preventive measures (face masks, social distancing) were maintained and continued [33].

In conclusion, the results of our pooled analysis of diagnostic accuracy of the fully-automated Roche Elecsys SARS-CoV-2 antigen ECLIA confirm that this test has considerably high cumulative diagnostic specificity and optimal diagnostic sensitivity for identifying nasopharyngeal samples with higher viral load. Further studies would be needed to define its role, placement and

cost-effectiveness with widespread strategies of SARS-CoV-2 testing. These results provide further evidence in support of the analytical, clinical and even technical advantages offered by laboratory-based, automated chemiluminescent antigen immunoassays compared to rapid antigen diagnostic tests in providing high-quality results of SARS-CoV-2 testing.

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