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Multicenter performance evaluation of the Elecsys HCV Duo immunoassay

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ABSTRACT

Background: The diagnostic accuracy of the Elecsys® HCV Duo antigen-antibody combination immunoassay (Roche Diagnostics GmbH) was evaluated for the detection of hepatitis C virus (HCV) infection, versus commercially available comparators.

Methods: This multicenter study (August 2020–March 2021) assessed the specificity of the Elecsys HCV Duo immunoassay and comparator assays in blood donor and routine clinical laboratory samples; sensitivity was determined in confirmed HCV-positive samples and seroconversion panels. The Elecsys HCV Duo immunoassay was compared with the Monolisa HCV Ag-Ab ULTRA V2, Murex HCV Ag/Ab Combination and ARCHITECT HCV Ag assays, as well as nucleic acid testing (NAT). The antibody (anti-HCV) module of the Elecsys HCV Duo immunoassay was compared with the Elecsys Anti-HCV II, Alinity s Anti-HCV, ARCHITECT Anti-HCV and RIBA HCV 3.0 SIA assays.

Results: The specificity of the Elecsys HCV Duo immunoassay was 99.94% (95% confidence interval [CI], 99.89–99.97) and 99.92% (95% CI, 99.71–99.99) in blood donor (n = 20,634) and routine clinical laboratory samples (n = 2531), respectively. The specificity of the Elecsys HCV Duo immunoassay was similar or better than comparator assays. The sensitivity of the Elecsys HCV Duo immunoassay in confirmed HCV-positive samples (n = 257) was 99.6%. In seroconversion panels, the Elecsys HCV Duo immunoassay detected infections earlier (2.2–21.9 days) than all but one of the comparator assays and detected HCV 1.8 days later than NAT.

Conclusions: The Elecsys HCV Duo immunoassay shows high diagnostic accuracy, reduces the diagnostic window, and could be used when NAT is not possible.

Abbreviations

Ag-Ab	antigen-antibody
Anti-HCV	antibodies to hepatitis C virus
Anti-HCV-/RNA+	anti-hepatitis C virus-negative/hepatitis C virus-ribonucleic acid-positive
Anti-HCV+/RNA-	anti-hepatitis C virus-positive/hepatitis C virus-ribonucleic acid-negative
Anti-HCV+/RNA+	anti-hepatitis C virus-positive/hepatitis C virus-ribonucleic acid-positive
CI	confidence interval
CE	Conformitè Europëenne
HCV	hepatitis C virus
HCV-Ag	hepatitis C virus core antigen
HIV	human immunodeficiency virus
NAT	nucleic acid testing
RNA	ribonucleic acid
qRT-PCR	quantitative reverse transcription polymerase chain reaction

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1. Introduction

Approximately 58 million people are living with chronic hepatitis C virus (HCV) infection worldwide, with an estimated 1.5 million new infections occurring annually [1]. Around 70% of people infected with HCV will develop chronic HCV infection, which can lead to liver cirrhosis and cancer if left untreated [1]. Early reliable detection of HCV is essential for management of patients with HCV infection and reducing the risk of HCV transmission by infected bodily fluids [2,3].

Serological assays for antibodies to HCV (anti-HCV) can identify people with current, resolved or treated HCV infection, while nucleic acid testing (NAT) for HCV ribonucleic acid (RNA) or an HCV core antigen (HCV-Ag) assay can be used to diagnose acute HCV infection, when antibodies may be undetectable [4]. HCV-Ag assays have a lower diagnostic sensitivity for HCV and a slightly longer diagnostic window (2–3 days) compared with NAT testing; however, the clinical utility of HCV-Ag assays has been demonstrated previously [5,6]. Furthermore, the European Association for the Study of the Liver recommends that an HCV-Ag assay can be used for the diagnosis of acute or chronic HCV infection instead of NAT [7].

Anti-HCV serological assays are generally used as first-line tests for the diagnosis of patients with suspected HCV infection and for screening purposes [8]. A major challenge in detecting HCV infection using anti-HCV assays is the long diagnostic window, during which a patient with HCV infection undergoes seroconversion and can test negative for anti-HCV, leading to a false-negative result if NAT or HCV-Ag testing is not performed in parallel [9]. This window can last 8 weeks after initial infection, or longer in patients who are severely immunocompromised (e.g., patients on hemodialysis) [9].

Fourth generation HCV antigen-antibody (Ag-Ab) combination assays could shorten the diagnostic window and take advantage of antibody detection in situations where HCV-Ag and HCV NAT might provide negative results in HCV-infected individuals or when HCV NAT is not available [10–12]. The Elecsys® HCV Duo immunoassay (Roche Diagnostics GmbH, Mannheim, Germany) is an electrochemiluminescence "ECLIA" immunoassay for the in vitro qualitative simultaneous detection of HCV-Ag and anti-HCV in human serum and plasma and is intended for use on cobas e immunoassay analyzers (Roche Diagnostics International Ltd, Rotkreuz, Switzerland). The Elecsys HCV Duo immunoassay is the first test to provide parallel, but separate, read-out of HCV-Ag and anti-HCV results compared with other combination assays that show a combined result only. Additionally, a final Elecsys HCV Duo result is calculated equal to the highest cut-off index value of the sub-results (HCV-Ag and anti-HCV). In conjunction with other laboratory tests and clinical information, the assay has potential as a first-line diagnostic and screening test for HCV infection in clinical laboratories and as a screening tool for blood products in blood donation centers. The sub-results are intended to aid the selection of the confirmatory testing algorithm for reactive samples. The Elecsys HCV Duo immunoassay is approved for clinical use in Conformitè Europëenne (CE)-marked countries.

This study evaluated the diagnostic accuracy of the Elecsys HCV Duo immunoassay in an international, multicenter study, and compared its performance to commercially available assays.

2. Materials and methods

2.1. Study design

The diagnostic accuracy of the Elecsys HCV Duo immunoassay on the cobas e 801 analyzer was evaluated at seven sites (August 2020–March 2021): four blood donation centers (Frankfurt and Hagen, Germany; Amsterdam, Netherlands; Johannesburg, South Africa) and one routine clinical diagnostic laboratory (Augsburg, Germany). Additional testing was conducted at TRIGA-S (Habach, Germany) and Roche Diagnostics (Penzberg, Germany).

The Elecsys HCV Duo immunoassay was compared with two commercially available Ag-Ab combination assays, the Monolisa HCV Ag-Ab ULTRA V2 (Bio-Rad Laboratories Inc, Marnes-la-Coquette, France) and the Murex HCV Ag/Ab Combination (DiaSorin, Saluggia, Italy), as well as the HCV-Ag only ARCHITECT HCV Ag assay (Abbott GmbH & Co. KG, Wiesbaden, Germany). The anti-HCV module of the Elecsys HCV Duo immunoassay was compared with the Elecsys Anti-HCV II (Roche Diagnostics International Ltd), Alinity s Anti-HCV (Abbott GmbH & Co. KG), ARCHITECT Anti-HCV (Abbott GmbH & Co. KG) and Chiron RIBA HCV 3.0 SIA (distributed by Ortho-Clinical Diagnostics, Raritan, NJ). The comparator assays and confirmatory methods used in the study are summarized in Table 1 and described in detail in the Supplementary Methods. *2.2. Ethics statement*

The study complied with the International Council for harmonisation Guidelines for Good Clinical Practice and the principles of the Declaration of Helsinki. The study protocol was submitted to the relevant ethics committees prior to study initiation. Ethical approval for the use of pseudonymized samples from blood donation centers was granted by the Ethik-Kommission des Fachbereichs Medizin der Goethe-Universität (Frankfurt), Ethik-Kommission der Ärztekammer Westfalen-Lippe (Hagen), Sanquin Blood Supply Ethics Advisory Council (Amsterdam), and the South African National Blood Service Human Research Ethics Committee (2020/0527; Johannesburg). Ethical approval was not necessary for residual, anonymized samples (routine clinical and HCV-confirmed positive samples).

2.2. Samples

All samples used in the study were residual serum or citrate/ethylenediaminetetraacetic acid-plasma samples that were pseudonymized (blood donor samples) or fully anonymized (routine clinical and HCV-confirmed positive samples) (Table 1).

2.3. Specificity analyses

For the specificity analyses, samples from first-time and repeat blood donors from four blood donation centers (Frankfurt, Hagen, Amsterdam, and Johannesburg) and routine clinical laboratory samples from one laboratory site (Augsburg) were used. Routine clinical laboratory samples included routine diagnostic samples from a variety of primary care facilities and hospitals, samples from pregnant women, and samples from patients on hemodialysis. Blood donor samples from the Frankfurt and Hagen sites were frozen and shipped to TRIGA-S (contract research services laboratory; Habach, Germany) for testing with the Monolisa HCV Ag-Ab ULTRA V2 assay; all other measurements were conducted at the respective study sites.

All samples that were positive using the Elecsys HCV Duo or a comparator assay were repeated according to the assay manufacturer's instructions or the established routine of the laboratory. At the blood donation centers, anti-HCV and nucleic acid testing (NAT; Procleix Ultrio Elite, Grifols Diagnostic Solutions, Inc., Emeryville, CA; or cobas® MPX, Roche Diagnostics GmbH) for HCV were performed in parallel. Anti-HCV tests for seropositive samples were repeated twice. Samples were identified as repeatedly reactive if at least two of the three results were positive. Repeatedly reactive samples that were NAT-negative were confirmed with immunoblot (INNO-LIA HCV Score, Fujirebio, Ghent, Belgium).

At the routine laboratory site, only serological testing was performed. All reactive samples were retested twice using the Elecsys HCV Duo and Monolisa HCV Ag-Ab ULTRA V2 assays. According to the laboratory's standard practice, initially reactive samples with the routine Elecsys Anti-HCV II immunoassay were only retested if the cut-off index was 0.9–10.0. Repeatedly reactive samples were confirmed with immunoblot (INNO-LIA HCV Score) and the ARCHITECT HCV Ag assay.

The reference tests for the calculation of specificity for the Elecsys HCV Duo and comparator assays in blood donor samples was NAT

Table 1
Samples, comparator assays and confirmatory methods used in the study.

Source of samples	Comparator assays tested	Confirmatory methods	Samples, *n*	Sample type
Specificity analyses				
Blood donation centers				
German Red Cross Blood Donor Service, Frankfurt, Germany	Elecsys Anti-HCV II Monolisa HCV Ag-Ab ULTRA V2[a]	NAT (cobas MPX) Immunoblot (INNO-LIA HCV Score)	5091	Fresh and frozen serum from blood donors
German Red Cross Blood Donor Service West, Hagen, Germany	Elecsys Anti-HCV II Monolisa HCV Ag-Ab ULTRA V2[a]	NAT (cobas MPX) Immunoblot (INNO-LIA HCV Score)	5483	Fresh and frozen EDTA-plasma from blood donors
Sanquin Blood Supply Foundation, Amsterdam, Netherlands	Alinity s Anti-HCV	NAT (cobas MPX) Immunoblot (INNO-LIA HCV Score)	5017	Fresh serum and EDTA-plasma from blood donors
South African National Blood Service, Johannesburg, South Africa	Alinity s Anti-HCV	NAT (Procleix Ultrio Elite) Immunoblot (INNO-LIA HCV Score)	5043	Fresh EDTA-plasma from blood donors
Routine laboratory				
Labor Augsburg, Augsburg, Germany	Elecsys Anti-HCV II Monolisa HCV Ag-Ab ULTRA V2	ARCHITECT HCV Ag assay Immunoblot (INNO-LIA HCV Score)	2531	Frozen serum from testing of routine diagnostic samples, pregnant women, and patients on hemodialysis
Sensitivity analyses				
National Institute of Blood Transfusion, Paris, France	Monolisa HCV Ag-Ab ULTRA V2[b]	qRT-PCR (COBAS TaqMan® HCV Quantitative[c])	257	Characterized HCV-positive frozen citrate-plasma
Biomex GmbH, Heidelberg, Germany SeraCare Life Sciences, Milford, MA ZeptoMetrix, Buffalo, NY	Monolisa HCV Ag-Ab ULTRA V2 Murex HCV Ag/Ab Combination ARCHITECT HCV Ag Elecsys Anti-HCV II ARCHITECT Anti-HCV RIBA HCV 3.0 SIA	Not applicable	85[d]	Seroconversion panels

The manufacturers of the comparator assays and confirmatory methods were as follows: Alinity s Anti-HCV (Abbott Laboratories, Wiesbaden, Germany); ARCHITECT HCV Ag assay (Abbott Laboratories); cobas MPX (Roche Diagnostics GmbH, Mannheim, Germany); cobas HCV (Roche Diagnostics GmbH); COBAS TaqMan® HCV Quantitative (Roche Diagnostics GmbH); Elecsys Anti-HCV II (Roche Diagnostics International Ltd); INNO-LIA HCV Score (Fujirebio, Ghent, Belgium); Murex HCV Ag/Ab Combination assay (DiaSorin, Saluggia, Italy); Monolisa HCV Ag-Ab ULTRA V2 assay (Bio-Rad Laboratories Inc, Marnes-la-Coquette, France); and Procleix Ultrio Elite (Grifols Diagnostic Solutions, Inc., Emeryville, CA).

[a] Samples were frozen and shipped to TRIGA-S (Habach, Germany) to be tested using the Monolisa HCV Ag-Ab ULTRA V2 assay.

[b] Samples were shipped to Roche Diagnostics (Penzberg, Germany) to be tested using the Elecsys HCV Duo immunoassay.

[c] Samples were previously characterized as HCV-positive with COBAS TaqMan® HCV Quantitative.

[d] 85 seroconversion panels with a total of 777 bleeds.

Ag, antigen; Ab, antibody; Anti-HCV, antibodies to hepatitis C virus; EDTA, ethylenediaminetetraacetic acid; HCV, hepatitis C virus; NAT, nucleic acid testing; qRT-PCR, quantitative reverse transcription-polymerase chain reaction.

(cobas MPX for all centers except Johannesburg, which used Procleix Ultrio Elite) and immunoblot (INNO-LIA HCV Score). In routine clinical laboratory samples (Augsburg), the reference test was the ARCHITECT HCV Ag assay and immunoblot (INNO-LIA HCV Score).

2.4. Sensitivity analyses

Samples confirmed to be HCV-positive using quantitative reverse transcription polymerase chain reaction (qRT-PCR; COBAS® TaqMan® HCV Quantitative test, Roche Diagnostics GmbH) from the National Institute of Blood Transfusion (Paris, France) were used for sensitivity analyses. As no cobas e 801 analyzer was available at the Paris site, confirmed HCV-positive samples (Paris) were frozen and shipped to Roche Diagnostics (Penzberg, Germany) for testing with the Elecsys HCV Duo. The reference test for the calculation of sensitivity for the Elecsys HCV Duo and Monolisa HCV Ag-Ab ULTRA V2 assays in the HCV-confirmed positive samples was qRT-PCR (COBAS® TaqMan® HCV Quantitative test).

Furthermore, vendor-purchased seroconversion panels were used in the sensitivity analyses. The diagnostic window of HCV immunoassays was evaluated in seroconversion panels, which included comparison with NAT testing using the cobas® HCV (Roche Diagnostics GmbH). Reactive results from HCV-positive samples were not repeated. Full details are provided in the Supplementary Methods.

2.5. Additional analyses for assessment of diagnostic accuracy

Samples that were confirmed positive for potentially interfering substances, as well as samples from different stages of HCV infection, different HCV genotypes, and same-day fresh HCV-positive samples, were also investigated (full details provided in the Supplementary Methods).

2.6. Statistical analysis

Specificity and sensitivity point estimates with 95% confidence intervals (CIs) were calculated.

3. Results

3.1. Specificity of the Elecsys HCV Duo immunoassay and comparator assays in blood donor samples

Across all blood donation centers, the specificity of the Elecsys HCV Duo immunoassay was 99.94% (95% CI, 99.89–99.97; n = 20,634; Table 2). All 13 false-positive results from the Elecsys HCV Duo immunoassay in blood donor samples were attributed to the HCV-Ag module and were negative by confirmatory NAT and immunoblot. In samples used for method comparison between Ag-Ab combination assays, the specificity of Elecsys HCV Duo was 99.96% (95% CI, 99.90–99.99; n = 10,574) versus 99.90% (95% CI, 99.81–99.95; n = 10,574) for the

Table 2
Specificity of the Elecsys HCV Duo immunoassay, the anti-HCV module of the Elecsys HCV Duo immunoassay and respective comparator assays y in blood donor samples.

Assay	Elecsys HCV Duo	Elecsys HCV Duo	Elecsys HCV Duo	Elecsys HCV Duo	Monolisa HCV Ag-Ab ULTRA V2	Elecsys HCV Duo anti-HCV module	Elecsys Anti-HCV II	Alinity s Anti-HCV
Site(s)	**All**	**Amsterdam, Frankfurt, and Hagen**	**Johannesburg**	**Frankfurt and Hagen**	**Frankfurt and Hagen**	**All**	**Frankfurt and Hagen**	**Amsterdam and Johannesburg**
Total samples tested	20,634	15,591	5043	10,574	10,574	20,634	10,574	10,060
NR samples	*20,618*	*15,582*	*5036*	*10,568*	*10,561*	*20,631*	*10,566*	*10,052*
Confirmed indeterminate	2/20,618	1/15,582	1/5036	1/10,568	1/10,561	2/20,631	1/10,566	0/10,052
Confirmed positive	0/20,618	0/15,582	0/5036	0/10,568	0/10,561	0/20,631	0/10,566	0/10,052
True negative	20,616	15,581	5035	10,567	10,560	20,629	10,565	10,052
Negative[a]	**20,629**	**15,588**	**5041**	**10,571**	**10,571**	**20,629**	**10,571**	**10,058**
RR samples	*16*	*9*	*7*	*6*	*13*	*3*	*8*	*8*
Confirmed positive	2/16	1/9	1/7	1/6	1/13	2/3	1/8	1/8
Confirmed indeterminate	1/16	1/9	0/7	1/6	1/13	1/3	1/8	1/8
Confirmed negative	13/16	7/9	6/7	4/6	11/13	0/3	6/8	6/8
Specificity,% (95% CI)[b]	**99.94 (99.89–99.97)**	**99.96 (99.91–99.98)**	**99.88 (99.74–99.96)**	**99.96 (99.90–99.99)**	**99.90 (99.81–99.95)**	**100.0 (99.98–100.0)**	**99.94 (99.88–99.98)**	**99.94 (99.87–99.98)**

[a] Negative = True negative + RR – confirmed negative samples.
[b] The reference tests for the calculation of specificity for the Elecsys HCV Duo and comparator assays in blood donor samples was NAT (cobas MPX for all centers except Johannesburg, which used Procleix Ultrio Elite) and immunoblot (INNO-LIA HCV Score).
CI, confidence interval; HCV, hepatitis C virus; NR, non-reactive; RR, repeatedly reactive.

Monolisa HCV Ag-Ab ULTRA V2 assay (Table 2). In samples tested with the anti-HCV assays, the specificity of the anti-HCV module of the Elecsys HCV Duo immunoassay was 100.0% (95% CI, 99.98–100.0; n = 20,634), versus 99.94% (95% CI, 99.88–99.98; n = 10,574) for the Elecsys Anti-HCV II and 99.94% (95% CI, 99.87–99.98; n = 10,060) for the Alinity s Anti-HCV assays (Table 2).

3.2. Specificity of the Elecsys HCV Duo immunoassay and comparator assays in routine clinical laboratory samples

The specificity of the Elecsys HCV Duo immunoassay in 1251 routine diagnostic samples was 99.92% (95% CI, 99.54–100.0) versus 99.75% (95% CI, 99.28–99.95) for the Monolisa HCV Ag-Ab ULTRA V2 assay (Table 3). The specificity of the anti-HCV module of the Elecsys HCV Duo immunoassay in the same samples was 99.92% (95% CI, 99.54–100.0) versus 99.75% (95% CI, 99.28–99.95) for the Elecsys Anti-HCV II immunoassay (Table 3).

In 1057 samples from pregnant women, the specificity of the Elecsys HCV Duo and Monolisa HCV Ag-Ab ULTRA V2 assays was the same: 100.0% (95% CI, 99.65–100.0) (Table 3). In the same samples, the specificity of the anti-HCV module of the Elecsys HCV Duo immunoassay was 100.0% (95% CI, 99.65–100.0) versus 99.81% (95% CI, 99.32–99.98) for the Elecsys Anti-HCV II immunoassay (Table 3).

In 223 samples from patients on hemodialysis, the specificity of the Elecsys HCV Duo and Monolisa HCV Ag-Ab ULTRA V2 assays was the same: 99.55% (95% CI, 97.52–99.99) (Table 3). In the same samples, the specificity of the anti-HCV module of the Elecsys HCV Duo immunoassay and the Elecsys Anti-HCV II immunoassay was also 99.55% (95% CI, 97.52–99.99) for both assays (Table 3).

For all routine clinical laboratory samples (n = 2531), the overall specificity of both the Elecsys HCV Duo immunoassay and the anti-HCV module of the Elecsys HCV Duo immunoassay was 99.92% (95% CI, 99.71–99.99), versus 99.84% (95% CI, 99.59–99.96) for the Monolisa HCV Ag-Ab ULTRA V2 and 99.76% (95% CI, 99.48–99.91) for the Elecsys Anti-HCV II assays, respectively (Supplemental Table 1). There was one sample that was a true positive for the Elecsys HCV Duo (positive for HCV-Ag) but was not detected by the Elecsys Anti-HCV II and Monolisa HCV Ag-Ab ULTRA V2 assays.

3.3. Sensitivity of the Elecsys HCV Duo and comparator immunoassays in HCV-confirmed positive samples

The sensitivity of the Elecsys HCV Duo and Monolisa HCV Ag-Ab ULTRA V2 assays was measured in 257 HCV-confirmed positive samples. All samples in the anti-HCV-positive/HCV-RNA-positive (Anti-HCV+/HCV-RNA+; n = 148) and anti-HCV-positive/HCV-RNA-negative cohorts (Anti-HCV+/HCV-RNA-; n = 90) were reactive using the Elecsys HCV Duo immunoassay (Table 4). For the anti-HCV-negative/HCV-RNA-positive cohort (Anti-HCV-/HCV-RNA+), 18 of the 19 samples were reactive, resulting in a sensitivity of 94.6% (Table 4). The overall sensitivity of the Elecsys HCV Duo immunoassay across all cohorts was 99.6% (Table 4). Using the Monolisa HCV Ag-Ab ULTRA V2 assay, all samples in the Anti-HCV+/HCV-RNA+ cohort (n = 148) were reactive. Two and eight samples were non-reactive in the Anti-HCV+/HCV-RNA- (n = 90) and Anti-HCV-/HCV-RNA+ (n = 19) cohorts, respectively (Table 4). The overall sensitivity of the Monolisa HCV Ag-Ab ULTRA V2 assay across all cohorts was 96.1% (Table 4).

3.4. Sensitivity of the Elecsys HCV Duo and comparator immunoassays in seroconversion panel analyses

The Elecsys HCV Duo immunoassay detected all of the seroconversion panels tested and 62.4% of bleeds (85 seroconversion panels, 777 bleeds, Table 5). The Elecsys HCV Duo immunoassay detected more seroconversion panels and antigen positive bleeds than comparator assays (Table 5). The Elecsys HCV Duo immunoassay detected an

Table 3
Specificity of the Elecsys HCV Duo immunoassay, the anti-HCV module of the Elecsys HCV Duo immunoassay and respective comparator assays in routine diagnostic samples and routine samples from pregnant women and patients on hemodialysis.

Assay	Routine diagnostic samples			Pregnant women			Patients on hemodialysis				
	Elecsys HCV Duo	Monolisa HCV Ag-Ab ULTRA V2	Elecsys HCV Duo anti-HCV module	Elecsys Anti-HCV II	Elecsys HCV Duo	Monolisa HCV Ag-Ab ULTRA V2	Elecsys HCV Duoanti-HCV module	Elecsys Anti-HCV II	Elecsys HCV Duo	Monolisa HCV Ag-Ab ULTRA V2	Elecsys HCV Duoanti-HCV module	Elecsys Anti-HCV II
Total samples tested	1251	1251	1251	1251	1057	1057	1057	1057	223	223	223	223
NR samples	*1219*	*1218*	*1220*	*1217*	*1055*	*1055*	*1055*	*1053*	*221*	*221*	*221*	*221*
Confirmed indeterminate	1/1219	1/1218	1/1220	0/1217	0/1055	0/1055	0/1055	0/1055	0/221	0/221	0/221	0/221
Confirmed positive	0/1219	1/1218	0/1220	0/1217	0/1055	0/1055	0/1055	0/1055	0/221	0/221	0/221	0/221
True negative	1218	1216	1219	1217	1055	1055	1055	1053	221	221	221	221
Negative[a]	**1219**	**1219**	**1220**	**1220**	**1055**	**1055**	**1055**	**1055**	**222**	**222**	**222**	**222**
RR samples	*32*	*33*	*31*	*34*	*2*	*2*	*2*	*4*	*2*	*2*	*2*	*2*
Confirmed positive	31/32	30/33	30/31	30/34	2/2	2/2	2/2	2/4	1/2	1/2	1/2	1/2
Confirmed indeterminate	0/32	0/33	0/31	1/34	0/2	0/2	0/2	0/4	0/2	0/2	0/2	0/2
Confirmed negative	1/32	3/33	1/31	3/34	0/2	0/2	0/2	2/4	1/2	1/2	1/2	1/2
Specificity,%	**99.92**	**99.75**	**99.92**	**99.75**	**100.0**	**100.0**	**100.0**	**99.81**	**99.55**	**99.55**	**99.55**	**99.55**
(95% CI)[b]	**(99.54–100.0)**	**(99.28–99.95)**	**(99.54–100.0)**	**(99.28–99.95)**	**(99.65–100.0)**	**(99.65–100.0)**	**(99.65–100.0)**	**(99.32–99.98)**	**(97.52–99.99)**	**(97.52–99.99)**	**(97.52–99.99)**	**(97.52–99.99)**

[a] Negative = True negative + RR – confirmed negative samples.
[b] The reference tests for the calculation of specificity for the Elecsys HCV Duo and comparator assays in all routine clinical laboratory samples was the ARCHITECT HCV Ag assay and immunoblot (INNO-LIA HCV Score).
CI, confidence interval; HCV, hepatitis C virus; NR, non-reactive; RR, repeatedly reactive.

Table 4
Sensitivity of the Elecsys HCV Duo immunoassay and the Monolisa HCV Ag-Ab ULTRA V2 assay in HCV-confirmed positive samples.

	Total samples, *N*	Elecsys HCV Duo		Monolisa HCV Ag-Ab ULTRA V2	
		Samples detected, *n*	Sensitivity, %[a]	Samples detected, *n*	Sensitivity, %[a]
Anti-HCV+/HCV-RNA+	148	148	100.0	148	100.0
Anti-HCV-/HCV-RNA+	19	18	94.8	11	57.9
Anti-HCV+/HCV-RNA-	90	90	100.0	88	97.8
Total	**257**	**256**	**99.6**	**247**	**96.1**

[a] The reference test for the calculation of sensitivity for the Elecsys HCV Duo and Monolisa HCV Ag-Ab ULTRA V2 assays in the HCV-confirmed positive samples was qRT-PCR (COBAS® TaqMan® HCV Quantitative test)
Anti-HCV, antibodies to HCV; HCV, hepatitis C virus; RNA, ribonucleic acid.

additional 136 bleeds compared with the Elecsys Anti-HCV II immunoassay (40 seroconversion panels, whereas eight panels were undetected by the comparator assay), an additional 107 bleeds compared with the Murex HCV Ag/Ab Combination assay (58 seroconversion panels, whereas 11 panels were undetected by the comparator), and an additional 65 bleeds compared with the Monolisa HCV Ag-Ab ULTRA V2 assay (19 seroconversion panels, whereas 11 panels were undetected by the comparator). Compared with the Monolisa HCV Ag-Ab ULTRA V2 and Murex HCV Ag/Ab Combination assays, the Elecsys HCV Duo immunoassay detected HCV infection 8.6 and 2.2 days earlier, respectively (Table 5). However, the Elecsys HCV Duo immunoassay detected HCV infection 2.3 days later than the ARCHITECT HCV Ag assay (Table 5). The Elecsys HCV Duo immunoassay detected HCV 1.8 days later versus NAT (48 seroconversion panels with negative bleeds; Table 6). The Monolisa HCV Ag-Ab ULTRA V2 and Murex HCV Ag/Ab Combination assays detected 81.0% and 42.9% of the 48 seroconversion panels, respectively, and detected HCV 10.3 and 3.4 days later, respectively, versus NAT. The ARCHITECT HCV Ag assay detected all seroconversion panels and detected HCV 0.4 days later versus NAT.

3.5. Additional analyses for assessment of diagnostic accuracy

The Elecsys HCV Duo immunoassay was tested in 204 samples containing potentially interfering substances, of which 11 samples were reactive (Supplemental Table 2). All but one of these 11 reactive samples were confirmed positive/indeterminate by immunoblot; the one sample that was reactive with the Elecsys HCV Duo immunoassay but negative with immunoblot was from a patient with chronic hepatitis B infection. No further clinical information was available for this patient.

Reactivity with the Elecsys HCV Duo immunoassay was assessed in a range of HCV samples. Mixed plasma and serum samples from different stages of HCV infection ($n = 386$), different HCV genotypes (types 1–6; $n = 100$), and from same-day fresh HCV-positive samples ($n = 52$) were consistently reactive with the Elecsys HCV Duo immunoassay (Supplemental Table 3).

4. Discussion

The anti-HCV module of the Elecsys HCV Duo immunoassay showed high specificity (100.0% in blood donor samples and 99.92% in routine clinical laboratory samples), with no false-positive results in over 20,000 blood donation samples. In the routine clinical laboratory samples, one

Table 5
Assessment of seroconversion panels using the Elecsys HCV Duo immunoassay and comparator assays.

	Elecsys HCV Duo immunoassay[a]	Elecsys HCV Duo immunoassay[a] versus comparator assays					
	All seroconversion panels	Monolisa HCV Ag-Ab ULTRA V2[b]	Murex HCV Ag/Ab Combination[b]	ARCHITECT HCV Ag[c]	Elecsys Anti-HCV II[d]	ARCHITECT Anti-HCV[d]	RIBA HCV 3.0 SIA[d]
Seroconversion panels tested, *N*	85	19	58	20	40	46	30
HCV-positive bleeds/total number of bleeds, *n* (%)	485/777 (62.4)	92/167 (55.1) versus 27/167 (16.2)	325/577 (56.3) versus 218/577 (37.8)	114/180 (63.3) versus 122/180 (67.8)	264/349 (75.6) versus 128/349 (36.7)	288/392 (73.5) versus 127/392 (32.4)	204/242 (84.3) versus 55/242 (22.7)
Number of seroconversion panels detected, *n* (%)	85 (100.0)	19 (100.0) versus 8 (42.1)	58 (100.0) versus 47 (81.0)	20 (100.0) versus 20 (100.0)	40 (100.0) versus 32 (80.0)	46 (100.0) versus 38 (82.6)	30 (100.0) versus 16 (53.3)
Combined number of days needed for the comparator assay to detect an initial positive result in each seroconversion panel (relative to Elecsys HCV Duo), *N*[e]	–	+69	+104	−45	+574	+818	+350
Average number of days for the comparator assay to detect a positive bleed (relative to Elecsys HCV Duo), *N*[e]	–	+8.6	+2.2	−2.3	+17.9	+21.5	+21.9

[a] HCV-positive results using the Elecsys HCV Duo were indicative of samples with a positive result from the HCV-Ag module or anti-HCV module or both modules.
[b] HCV-positive results using the Monolisa HCV Ag-Ab ULTRA V2 and Murex HCV Ag/Ab Combination assays were indicative of samples with an overall positive result as these assays do not report HCV-Ag or anti-HCV results independently.
[c] HCV-positive results using the ARCHITECT HCV Ag were indicative of samples positive for HCV-Ag only.
[d] HCV-positive results using the Elecsys Anti-HCV II, ARCHITECT Anti-HCV or the RIBA HCV 3.0 SIA were indicative of samples positive for anti-HCV only.
[e] The first positive bleed for Elecsys HCV Duo was used as the reference point to calculate the relative time difference between Elecsys HCV Duo and the comparator assays to detect a positive bleed. Seroconversion panels that were not detected by the respective comparator assay were excluded from the analyses.
Anti-HCV, antibodies to HCV; HCV, hepatitis C virus; HCV-Ag, hepatitis C virus core antigen.

Table 6
Comparison of Elecsys HCV Duo immunoassay and comparator assays with NAT testing in the assessment of seroconversion panels.

	cobas HCV versus serologic HCV assay panel						
	Elecsys HCV Duo[a]	Monolisa HCV Ag-Ab ULTRA V2[b]	Murex HCV Ag/Ab Combination[b]	ARCHITECT HCV Ag[c]	Elecsys Anti-HCV II[d]	ARCHITECT Anti-HCV[d]	RIBA HCV 3.0 SIA[d]
Seroconversion panels tested, *N*	48	14	42	15	15	19	8
Number of seroconversion panels detected, *n* (%)	48 (100.0) versus 48 (100.0)	14 (100.0) versus 6 (42.9)	42 (100.0) versus 34 (81.0)	15 (100.0) versus 15 (100.0)	15 (100.0) versus 7 (46.7)	19 (100.0) versus 11 (57.9)	8 (100.0) versus 4 (50.0)
Combined number of days needed to detect an initial positive result in each seroconversion panel, *N*[e]	588 versus 674	44 versus 106	369 versus 485	238 versus 244	162 versus 337	267 versus 535	117 versus 217
Average number of days for the HCV assay panel to detect a positive bleed (relative to cobas HCV), *N*[e]	+1.8	+10.3	+3.4	+0.4	+25.0	+24.4	+25.0

A sub-cohort of seroconversion panels were selected where subsequent blood sampling turned from negative NAT bleeds to positive NAT bleeds.
[a] HCV-positive results using the Elecsys HCV Duo were indicative of samples with a positive result from the HCV-Ag module or anti-HCV module or both modules.
[b] HCV-positive results using the Monolisa HCV Ag-Ab ULTRA V2 and Murex HCV Ag/Ab Combination assays were indicative of samples with an overall positive result as these assays do not report HCV-Ag or anti-HCV results independently.
[c] HCV-positive results using the ARCHITECT HCV Ag were indicative of samples positive for HCV-Ag only.
[d] HCV-positive results using the Elecsys Anti-HCV II, ARCHITECT Anti-HCV or the RIBA HCV 3.0 SIA were indicative of samples positive for anti-HCV only.
[e] The last negative NAT bleed of a panel was used as Day 0 to calculate the relative time difference between NAT testing and the HCV assay panel to detect a positive bleed. Seroconversion panels that were not detected by the respective comparator assay were excluded from the analyses.
Anti-HCV, antibodies to HCV; HCV, hepatitis C virus; HCV-Ag, hepatitis C virus core antigen; NAT, nucleic acid test.

true positive sample detected by the Elecsys HCV Duo (positive for HCV-Ag) was not detected by the Elecsys Anti-HCV II or Monolisa HCV Ag-Ab ULTRA V2 assays. Since the latter is also designed for antigen detection, the sample was designated as a false-negative for the Monolisa HCV Ag-Ab ULTRA V2. The result was confirmed positive by the ARCHITECT HCV-Ag assay.

The Elecsys HCV Duo immunoassay showed high sensitivity (99.6% across all cohorts in HCV-confirmed positive samples) and detected all but one sample that was previously confirmed HCV-positive. The non-reactive sample with the Elecsys HCV Duo immunoassay corresponded to an early infection phase with a low viral load (8050 IU/mL). Furthermore, the Elecsys HCV Duo immunoassay detected HCV only 1.8 days later versus NAT. The Monolisa HCV Ag-Ab ULTRA V2 and Murex HCV Ag/Ab Combination assays detected HCV 10.3 and 3.4 days later, respectively, versus NAT, and the ARCHITECT HCV Ag assay, which was used for confirmation, detected HCV 0.4 days later versus NAT.

Fourth generation Ag-Ab combination HCV assays are more user-friendly and cost-efficient than NAT [13,14]; thus, demonstrating potential in low-income countries where NAT is unavailable and HCV screening is often conducted by rapid tests [15]. The diagnostic accuracy of the Elecsys HCV Duo immunoassay reported in this study is comparable with the sensitivity and specificity of NAT for detection of HCV

infection. Therefore, the benefit of additional NAT testing may be limited, especially in countries with a low prevalence of the disease and access to highly effective anti-viral medication for HCV. Similarly, the emergence of fourth generation combination assays for human immunodeficiency virus (HIV) [16] and hepatitis B virus [17] could offer improvements in quality-adjusted life years versus NAT, warranting future health economic analyses. This study also indicates that the Elecsys HCV Duo immunoassay is unaffected by potentially interfering antibodies to HIV, which was an issue with previous generation anti-HCV assays and in countries with a high prevalence of HIV and HCV co-infection [14,18,19].

A key strength of this study is the international, multicenter design that included a large number of samples, including blood donor specimens. Additionally, the Elecsys HCV Duo immunoassay allows readout of separate antigen and antibody results in parallel, whereas other Ag-Ab combination tests show a combined test result only. One limitation of this study is that there is no specific HCV-Ag neutralization method; consequently, the HCV-Ag module of the Elecsys HCV Duo immunoassay was not singularly tested. Future studies should assess the Elecsys HCV Duo immunoassay in other populations, such as patients with HIV, and assess its specificity and sensitivity as a screening tool in the general population.

5. Conclusion

The Elecsys HCV Duo immunoassay shows high specificity and sensitivity and could therefore be used in countries where NAT is not available. The Elecsys HCV Duo immunoassay showed the greatest reduction in diagnostic window versus other high throughput assays. An earlier diagnosis of HCV, alongside highly effective anti-viral medication, is crucial in eliminating the virus by 2030, as outlined by the World Health Organization [20].

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Role of the funding source

Roche Diagnostics International Ltd was involved in the study design, collection, analysis, and interpretation of data, writing of the manuscript, and the decision to submit the article for publication.

Informed consent

For all blood donation centers, blood donors are asked to give their consent with every donation that residual blood can be used for research purposes; therefore, there was no need for specific ethical approval for the use of pseudonymized leftover samples. For routine clinical laboratory and HCV-confirmed positive samples, informed consent was not required for the use of anonymized leftover material. For vendor-purchased seroconversion panels, details regarding informed consent are available from the respective vendor.

Data availability

This study was conducted in accordance with applicable regulations. Ethical approval was obtained for the blood donation centers; for Labor Augsburg and the National Institute of Blood Transfusion, samples were completely anonymized and, thus, ethical approval was not necessary. As the study data spanned multiple sites globally, we cannot refer data collection to a single ethics committee. Qualified researchers may request access from the following ethical committees:

1 Ethik-Kommission des Fachbereichs Medizin der Goethe-Universität (Frankfurt, Germany); protocol RD003540; email: m.schmidt@-blutspende.de
2 Ethik-Kommission der Ärztekammer Westfalen-Lippe (Hagen, Germany); protocol RD003540; email: ethik-kommission@aekwl.de
3 Sanquin Blood Supply Ethics Advisory Council (Amsterdam, The Netherlands); protocol RD003540; email: e.bakker@sanquin.nl
4 South African National Blood Service Human Research Ethics Committee (2020/0527; Johannesburg, South Africa); protocol RD003540; email: SRCAdmin@sanbs.org.za.

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Short description of paper

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Declaration of Competing Interest

ER has previously received speaker honorarium from F. Hoffmann-La Roche Ltd, which was independent from this study. RB and MK are employees of Roche Diagnostics GmbH and hold non-voting equity security in F. Hoffmann-La Roche Ltd. The remaining authors have no competing interests to declare.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcv.2022.105293.

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