Research Article

Evaluation of Anti-HCV, HBsAg and HAV IgM on Three Different Chemiluminescence Immunoassay Systems for Hepatitis Screening

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Keywords

Analytical sensitivity, Anti-HCV; Chemiluminescent immunoassay, CLIA, Clinical sensitivity, Clinical specificity, Donor screening, Early infection detection, HAV IgM, Hepatitis serology, HBsAg, Performance evaluation study, Seroconversion, Viral hepatitis elimination

Abstract

Background: Reliable screening for hepatitis serological markers is critical for early diagnosis, patient management, and transfusion safety, as acute infections are often asymptomatic. Thus, it is difficult to determine an infection by a clinical assessment alone, often remaining undiagnosed for years, leading to delayed treatment, disease progression and ongoing transmission. With this in mind, HBsAg, Anti-HCV, and HAV IgM serve as the foundational, first-line assays in the diagnostic and screening algorithms for these viruses. In support global viral hepatitis elimination goals, evaluations of commercially available hepatitis assays are critical to ensure they possess the sensitivity required for accurate laboratory detection, particularly during the early stages of infection when analyte levels are low.
Objectives: To perform an analytical performance evaluation of three commercial hepatitis tests, specifically assessing their capability to detect early seroconversion and low-level reactivity.

Methods: Anti-HCV (Antibody to Hepatitis C Virus), HBsAg (Hepatitis B Surface Antigen) and HAV IgM (IgM antibody to Hepatitis A virus) on three CLIA analytical systems were evaluated on three CLIA analytical systems: Alinity i (Abbott Laboratories Inc., Abbott Park, Illinois, USA), Maglumi X3 (Shenzhen New Industries Biomedical Engineering Co., Ltd., Shenzhen, China), and CL-1200i (Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China).

Study design: Analytical precision, analytical sensitivity, endogenous interferences of common metabolites, clinical sensitivity and clinical specificity were evaluated using reference materials, seroconversion panels and leftover patient samples, confirmed by nucleic acid amplification testing (PCR).

Results: Alinity i demonstrated the highest analytical sensitivity for HBsAg, remaining reactive at the lowest concentration evaluated (0.032 IU/mL), with complete (100%) clinical sensitivity across all assays. Maglumi X3 showed comparable HBsAg analytical sensitivity (0.043 IU/mL), very good clinical sensitivity (100% for Anti-HCV, 97.37% for HBsAg, 99% for HAV IgM), and high

specificity. CL-1200i exhibited reduced HBsAg analytical sensitivity (0.065 IU/mL), lower clinical sensitivity (91% for Anti-HCV, 84.87% for HBsAg, 92% for HAV IgM), with delayed seroconversion detection relative to Alinity i and Maglumi X3. All systems demonstrated high clinical specificity (>97.8%), although CL-1200i showed a higher rate (2.2% for Anti-HCV; 0.6% for HBsAg; 1.67% for HAV IgM) of false-reactivity relative to Alinity i (0.2% for Anti-HCV; 0.2% for HBsAg; 0.33% for HAV IgM) and Maglumi X3 (1.2% for Anti-HCV; 0.13% for HBsAg; 0.67% for HAV IgM).

Conclusions: Significant analytical and clinical performance differences exist among the three CLIA analytical systems evaluated in this study. This study provides analytical performance information that should aid medical laboratories when selecting a system for early infection detection and blood safety screening. Post-market evaluations of infectious disease immunoassays are increasingly critical under current IVDR requirements, particularly for early seroconversion detection where small analytical differences may translate into clinically relevant diagnostic delays and increased risk of missed infections.

Introduction

Hepatitis A virus (HAV), hepatitis B virus (HBV), and hepatitis C virus (HCV) infections remain major global public health concerns and continue to pose a significant risk to blood safety [1–3]. Despite substantial advances in screening strategies, transfusion-transmitted hepatitis remains a critical challenge, particularly in high throughput screening environments where serological testing represents the first line barrier to transmission [4,5]. Because acute hepatitis infections are frequently asymptomatic and cannot be reliably distinguished clinically, laboratory testing plays a central role in both individual patient management and population prevention strategies [4,6,7].

Blood donor screening programs rely on sensitive serological assays for the detection of hepatitis B surface antigen (HBsAg) and antibodies to HCV (anti-HCV) to prevent the transmission of chronic viral hepatitis [4,8]. Assay performance near clinical decision thresholds is of particular importance, as low level antigenemia or early seroconversion may occur during the early infection window or in cases of occult HBV or HCV infection [9]. Failure to detect such low reactivity samples may result in undetected infectious donations, with direct consequences for transfusion safety and public health [10].

Conversely, insufficient assay specificity may lead to false reactive results, unnecessary donor deferral, and increased confirmatory testing burden [8]. Detection of immunoglobulin M antibodies to HAV (HAV IgM) is central to the diagnosis of acute hepatitis A and to differentiating the infection from other causes of acute viral hepatitis [11]. Reliable HAV IgM testing is therefore essential for timely patient management, outbreak control, and epidemiological surveillance, particularly in emergency and outbreak driven testing settings [12,13].

Early and accurate laboratory detection of hepatitis A, B, and C infections is also fundamental to achieving global hepatitis control and elimination targets [1,2]. The World Health Organization (WHO) has emphasized that delayed or missed detection of hepatitis virus markers (particularly at low analyte concentrations) may contribute to ongoing transmission and delayed treatment initiation, thus undermining elimination efforts [2]. Consequently, analytical sensitivity, precision, and specificity at or near assay cutoff values represent clinically relevant performance characteristics for hepatitis serological assays used in routine diagnostic and blood screening practice [6,14].

Automated chemiluminescent immunoassay (CLIA) analytical systems are widely implemented for blood screening and infection detection in clinical laboratories due to their high throughput, automation, and standardized workflows[15]. However, differences in assay design, detection principles, and calibration approaches may lead to variability in analytical and clinical performance, particularly for low reactivity samples and early infection stages [16,17]. While numerous studies have evaluated individual hepatitis immunoassays, comparisons of widely used CLIA analytical systems across multiple hepatitis markers remain limited. In this context, real world performance evaluations are essential to characterize assay behaviour beyond manufacturer validation studies conducted under factory-controlled conditions [18,19].

The present study aims to provide a comprehensive analytical and clinical performance comparison of three automated CLIA analytical systems (Alinity i, Maglumi X3, and CL-1200i) commonly used for hepatitis serological screening. Specifically, we evaluated the analytical precision, analytical sensitivity, endogenous interference, and clinical sensitivity and specificity for Anti-HCV (Antibody to Hepatitis C Virus), HBsAg (Hepatitis B Surface Antigen), and HAV IgM (IgM antibody to Hepatitis A virus) under routine laboratory conditions.

Materials and Methods

Study design

This research was conducted as a head-to-head comparative evaluation of three automated chemiluminescent immunoassay systems. The study employed a cross-sectional experimental design to assess and compare the analytical and clinical performance of the Alinity i, Maglumi X3, and CL-1200i systems. To ensure a rigorous comparison, all systems were evaluated simultaneously using identical sets of reference materials, seroconversion panels, and patient serum samples under routine clinical laboratory conditions.

All measurements were performed by trained laboratory personnel using standard operating procedures, routine calibration, and manufacturer recommended settings to reflect a real-world diagnostic environment. Residual serum samples were prospectively collected after completion of routine diagnostic testing and were fully de-identified prior

to analysis. Samples were stored at -20 °C until testing.
The study was conducted at a private clinical laboratory in Bucharest, Romania, where all three analytical platforms were co-located. Due to institutional confidentiality policies, the specific name of the facility has been withheld. All procedures used fully de-identified residual serum samples collected after the completion of routine diagnostic testing. In accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines and the international standard ISO 15189:2022 [21], clinical laboratories must verify manufacturer performance claims using available relevant materials, including residual clinical specimens prior to the implementation of any system for patient reporting. Under the institutional and national regulatory standards in Romania, quality assurance evaluations using anonymized leftover specimens do not constitute interventional human subjects research. As such, the study was exempt from formal Ethics Committee approval and the requirement for informed consent. All data handling and procedures were conducted in strict adherence to the principles of the Declaration of Helsinki [20], CLSI guidelines, and in strict accordance with the instructions for use provided by the manufacturers.

Immunoassays and CLIA Devices

This study evaluated three automated CLIA analytical systems utilizing different chemiluminescent technologies. The Alinity i (Abbott Laboratories Inc., Abbott Park, Illinois, USA) and Maglumi X3 (Shenzhen New Industries Biomedical Engineering Co., Ltd., Shenzhen, China) utilize direct chemiluminescence (flash light production). These systems use luminescent labels attached directly to the antigen or antibody, resulting in fast reaction kinetics. In contrast, the CL-1200i (Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China) employs indirect chemiluminescence via glow light production. This system employs an enzyme-catalyzed reaction, typically utilizing an alkaline phosphatase label for a sustained light emission. Technical specifications for each CLIA system are summarized in Supplementary Table 1 and detailed information regarding the specific analytical and clinical parameters evaluated for Anti-HCV, HBsAg, and HAV IgM is presented in Table 1.

Table 1: The study protocol and materials used.

Assay	Precision	Analytical Sensitivity	Endogenous Interferents	Clinical Sensitivity	Clinical Specificity
AntiHCV	5 days (5 reps x 5 days) / Internal quality controls	N/A	Creatinine (15 mg/dL), Glucose (1000 mg/dL), Glycerol (18 mg/dL), Lactate (90 mg/dL), Pyruvate (5 mg/dL), Blood Urea Nitrogen (120 mg/dL), Uric Acid (25 mg/dL)	100 positive samples vs PCR	500 negative samples (50 pregnant females)
HBsAg		2nd WHO IS HBsAg (NIBSC 00/588)		250 samples (98 panel samples: low positive, occult, seroconversion; 152 positive patient samples vs PCR)	1500 negative samples
HAV IgM		N/A		100 positive samples	300 negative samples

N/A (Not applicable).

Analytical precision

The overall precision (within-lab precision) was defined as a combination of the within-run and between-run variances. The evaluation was conducted using manufacturer-provided internal quality controls according to the the Clinical and Laboratory Standards Institute CLSI EP15-A3 guidelines [21]. The protocol involved 5 replicates in a single run per day over 5 consecutive days, totaling 25 replicates for each quality control material.

Analytical sensitivity

Analytical sensitivity was defined as the lowest concentration of the WHO Reference Panel (NIBSC code 03/262) for HBsAg that yielded reactive results across replicate measurements on the analytical systems. Briefly, the panel consists of a series of fourfold dilutions (up to 1/256) of the 2nd WHO IS for HBsAg genotype A2, subtype group adw2 (NIBSC 00/588) [22]. The reference panel is comprised of four positive samples (panel members A-D) and one negative control sample (panel member E; normal re-calcified plasma).
Initial sensitivity testing was performed using panel members A-D and E (the negative control). To further characterize assay performance at low HBsAg concentrations near the detection limit, additional dilutions of panel member D (1/256 of IS 00/588) were prepared, generating three supplementary concentrations (D1-D3). Panel member E was used as matrix material to prepare additional dilutions of panel member D (1/256 of IS 00/588). Assigned concentrations for each dilution were calculated based on the assigned unitage of the 2nd WHO International Standard (33 IU/mL). These extended dilutions were included to allow comparative assessment of analytical sensitivity beyond the standard panel range. The final extended dilution was determined when two out of the three CLIA assay systems failed to detect the diluted sample. All panel members

and additional dilutions were tested on the same day on each analyzer, with five replicate measurements per concentration. Analytical sensitivity in this study was reported as a comparative performance characteristic and does not represent a formal limit of detection (LoD) determination.

Endogenous interferences

Analytical interference for each assay was evaluated with seven common endogenous metabolites expected in the patient population at high concentrations using the ASSURANCE™ Interference Test Kit INT-06 (Sun Diagnostics, LLC, New Gloucester, Maine, USA). This kit contained the following metabolites, with targeted concentrations shown in parentheses: creatinine (15 mg/dL), glucose (1000 mg/dL), glycerol (18 mg/dL), lactate (90 mg/dL), pyruvate (5 mg/dL), blood urea nitrogen (120 mg/dL), uric acid (25 mg/dL). Leftover patient serum samples without any visible lipemia, haemolysis or icterus were used to prepare negative, low positive and high positive analyte pools. Serum pools were tested before spiking to establish the baseline measurand concentrations, then test pools were prepared by adding the interferent material to bring the test pool to the target interferent concentration (Table 1). The required amount of interferent materials were calculated using the Assurance™ Excel Worksheets [23]. For each test pool, a matching control pool with the same volume of solvent (purified water) as used in preparation of the corresponding interferent pool was prepared. Same day measurements for each potential interferent were performed by measuring 6 replicates per run for each sample pool on all the analyzers. Furthermore, to minimize experimental error, the replicates were analyzed in alternating pattern between control pools and test pools. Because the evaluated immunoassays generate quantitative results that are interpreted qualitatively, interference acceptability was assessed relative to the assay cutoff rather than fixed percentage bias limits. Specifically, the maximum allowable difference (dmax) was established relative to the medical decision point (immunoassay cutoff). For negative samples pool, dmax was defined as the maximum allowable signal shift that would not cause a true negative result to exceed the positivity cutoff. For the positives and the weak positives pools, dmax was defined as the maximum allowable signal shift that would not reduce a true positive result below the cutoff.

Clinical sensitivity

Clinical sensitivity evaluation was conducted separately for each assay, using a combination of leftover patient samples and commercially available seroconversion and sensitivity panels (Table 1). Anti-HCV clinical sensitivity was assessed using 100 positive leftover patient samples, confirmed by HCV RNA PCR. HAV IgM clinical sensitivity was evaluated using 100 positive leftover patient samples, classified as positive based on repeated reactivity, defined as duplicate reactive results obtained on at least two of the three evaluated analyzers (as per manufacturer instructions). HBsAg clinical sensitivity was evaluated by testing a total of 250 samples (comprising 98 panel samples and 152 positive leftover patient samples). Furthermore, the positive leftover samples were also confirmed positive by HBV DNA PCR for validation.
Seroconversion performance was assessed using six commercially available HBsAg seroconversion panels 0605-0038; 0605-0039; 0605-0041; 0605-0059 (SeraCare, LCG Group, LCG Limited, Guildford, UK [24]), and 67303; 65449 (ZeptoMetrix LLC, Franklin, Massachusetts, USA [25]), consisting of sequential plasma samples collected from individual donors during HBV seroconversion. Additional sensitivity assessment was performed using AccuSet Low Titre HBsAg Performance Panel 0805-0417; AccuSpan Sensitivity Panel HBsAg Subtype 2410-0161 (SeraCare, LCG Group, LCG Limited, Guildford, UK). The low titre performance panel consisted of undiluted HBV positive plasma samples with reactivities close to the cutoff signal of these commercial HBsAg assays. The sensitivity panel consists of two series of dilutions for HBsAg subtypes ad and ay, designed to evaluate low end assay sensitivity. The panel manufacturer tested the WHO HBsAg subtype adw2, genotype A Reference Panel (NIBSC code 03/262) in the same run as the AccuSpan HBsAg Sensitivity Panel members [24].

Clinical specificity

Clinical specificity evaluation was conducted separately for each assay using leftover negative patient samples. The varying sample sizes across the evaluated markers reflect the practical challenges of prospectively acquiring sufficient volumes of adequately characterized uncontaminated leftover diagnostic specimens during the study period. Based on this sample availability, evaluations were performed as follows: Anti-HCV using 500 negative patient samples (including 50 samples obtained from pregnant women); HBsAg using 1,500 negative patient samples; and HAV IgM using 300 negative patient samples. Reactive (positive) results detected for Anti-HCV, HBsAg, and HAV IgM assays were further investigated using confirmatory nucleic acid amplification testing together with clinical information (where available) to determine whether an active infection was present or if the result was falsely reactive.

Statistical analysis

Analyses were performed to evaluate the comparative performance of the three analytical systems. Precision was quantified using the coefficient of variation (CV%) and analyzed via analysis of variance (ANOVA, using the day of the run as random factor variable) to determine within run and total within laboratory signal stability. For clinical sensitivity and specificity, point estimates were calculated as proportions of correctly identified samples, with 95% confidence intervals (CI) determined using the exact binomial Clopper-Pearson method.

Table 2: Repeatability (CV%) results compared to the manufacturer's claimed values.

Assay	Control sample	N	Alinity i		Maglumi X3		CL1200i	
			Claimed	Measured	Claimed	Measured	Claimed	Measured
AntiHCV	Negative Control	25	7.00%	5.35%	13.24%	5.12%	8.98%	9.83%
	Positive Control	25	3.20%	1.48%	4.22%	3.89%	5.86%	4.82%
HBsAg	Negative Control	25	N/A	7.08%	N/A	14.69%	8.00%	10.70%
	Positive Control	25	3.20%	2.07%	4.71%	3.54%	4.49%	7.49%
	Positive Control 2	25	N/A	N/A	4.66%	3.23%	N/A	N/A
HAV IgM	Negative Control	25	7.00%	6.80%	N/A	8.08%	3.41%	4.17%
	Positive Control	25	6.30%	3.39%	1.96%	3.90%	2.25%	4.55%

N/A (Not available). Green shading indicates measured CV% values meeting or exceeding manufacturer performance claims (≤ claimed CV%). Red shading indicates measured CV% values higher than the manufacturer's claimed precision.

Regarding endogenous interference studies, patient serum samples were pooled to create standardized negative, low positive, and high positive analyte levels. Pooling was necessary to provide sufficient sample volume for multi replicate testing and to establish a stable baseline measurand concentration before the addition of interferent materials. The acceptability of interference was assessed using the maximum allowable difference (dmax) relative to the medical decision point (assay cutoff). Finally, the robustness of each system against false reactivity was assessed by measuring the statistical separation between the negative sample distribution and the assay cutoff, expressed in standard deviations (SD).

Results

Analytical precision results

The analytical precision of the three evaluated CLIA assay systems was determined by assessing both within-run repeatability and total within-laboratory reproducibility across all three hepatitis markers (Anti-HCV, HBsAg, and HAV IgM). Precision was computed using the coefficient of variation (CV%) across multiple replicates of multi-level control materials. Statistical parameters for precision are summarized in Table 2 (exhaustive statistical parameters shown in Supplementary Table 2).

The Alinity i platform exhibited the best relative precision metrics throughout the evaluation. For this system, the measured CV values consistently fell well below the manufacturer's claimed limits (see Table 2). This robust signal stability was uniformly maintained across all evaluated assays and across both negative and positive control levels.
The Snibe Maglumi X3 and Mindray CL-1200i systems exhibited higher CV percentages compared to the Alinity i [26]. Specifically, higher imprecision (elevated CV percentages, see Table 2) was observed on both systems when evaluating negative control materials at concentrations near the assay cutoff thresholds.
At the positive control levels, both the Alinity i and Maglumi X3 systems demonstrated CVs below their claimed limits, whereas the CL-1200i exhibited comparatively higher CVs. For these assay specific controls, both the Alinity i and Maglumi X3 systems demonstrated good signal stability (see Supplementary Table 2), whereas the CL-1200i exhibited comparatively higher (though still acceptable) precision and signal fluctuation.

Analytical sensitivity results for HBsAg

Analytical sensitivity results for the HBsAg assays are summarized in Table 3. All three systems measured reactive results across the panel members A-D, corresponding to HBsAg concentrations from 8.25 IU/mL to 0.13 IU/mL. At lower concentrations generated by additional dilutions of panel member D, differences in analytical sensitivity were observed between the evaluated systems. Alinity i remained reactive across all tested dilutions, including the lowest concentration evaluated (0.032 IU/mL; D3), with consistent reactivity observed across all five replicate measurements. Maglumi X3 remained reactive at 0.065 IU/mL (D1) and at 0.043 IU/mL (D2). At the 0.043 IU/mL dilution, three out of five replicate measurements were above the assay cutoff (≥0.05 IU/mL), while two replicates were below the cutoff. According to the manufacturer's instructions for use, this pattern was classified as reactive. At the lowest concentration tested (0.032 IU/mL; D3), all replicate measurements were non-reactive. CL-1200i remained reactive at 0.065 IU/mL (D1). At 0.043 IU/mL (D2), one out of five replicate measurements exceeded the assay cutoff, while the remaining replicates were below the cutoff; according to the manufacturer's instructions for use, the result was classified as non-reactive. At 0.032 IU/mL (D3), all replicate measurements were non-reactive.

Table 3: HBsAg analytical sensitivity comparison. WHO Reference Reagent HBsAg subtype adw2, genotype A, WHO Reference Panel (NIBSC 03/262): Dilutions of the 2nd WHO International Standard for HBsAg genotype A2 subtype group adw2 (NIBSC 00/588) Assigned unitage: 33 IU/mL.

Panel members	Dilution unitage (IU/mL)	Alinity i (S/CO)	Maglumi X3 (IU/mL)	CL1200i (IU/mL)
A (1/4 IS)	8.25	929.282	10.231	9.291
B (1/16 IS)	2.06	331.676	2.775	3.549
C (1/64 IS)	0.52	126.550	0.400	0.793
D (1/256 IS)	0.13	32.910	0.198	0.106
D1 (1/2 D)	0.065	12.978	0.056	0.073
D2 (1/3 D)	0.043	8.782	0.051	0.048*
D3 (1/4 D)	0.032	6.618	0.023	0.039
E (Negative)	0	0.200	0.023	0.011

*Positive results: green highlight, red line: detection limit; *1 reactive result out 5 replicates tested, the result is considered negative. According to the instructions for use: Samples with HBsAg concentration ≥0.05 IU/mL are considered Initially Reactive. Initially Reactive samples must be retested in duplicate, if both results are less than 0.05 IU/mL, the sample is non-reactive; no further test is needed for the patient.*

Endogenous interference evaluation results

No clinically significant interference was observed from common endogenous substances tested according to CLSI EP07 for all three CLIA systems and infectious disease immunoassays (Data not shown). Differences were observed (in some cases exceeding ±10% relative difference between control and test pools) for some runs but this did not alter the qualitative interpretation of results (positive/negative), as no result crossed the assay cutoff. Overall, Snibe Maglumi X3 immunoassays demonstrated slightly higher signal variation compared to Alinity i and CL-1200i. Notably, for Anti-HCV, Maglumi X3 showed significant concentration differences of 10.1-25.2% for creatinine (15 mg/dL) across negative, low positive, and positive pools, while CL-1200i showed differences of 5.3-10.4% for uric acid (25 mg/dL). For HBsAg, Maglumi X3 showed differences of 5.1-17.6% for lactate (90 mg/dL), while CL-1200i showed differences of 4.3-10.7% for lactate (90 mg/dL). For all other endogenous metabolites, signal differences remained within ±10%. Accordingly, interference effects exceeding ±10% were considered analytically but not clinically significant for qualitative interpretation [27].

Clinical sensitivity evaluation results

Clinical sensitivity evaluation results for Anti-HCV

Alinity i and Maglumi X3 demonstrated a clinical sensitivity of 100%, correctly identifying all 100 positive samples, with no false negative results observed (Table 4, with results distribution shown in Supplementary Figure 1A). In contrast, CL-1200i correctly classified 91 of 100 positive samples, corresponding to a clinical sensitivity of 91.0% (95% CI: 83.77-95.19%), with 9 false negative results. The majority of reactive samples on all systems were distributed well above the assay cutoff (S/Co, AU/mL, or COI = 1). For CL-1200i, false negative results were predominantly associated with samples exhibiting low signal values close to the cutoff (on the other systems), indicating reduced sensitivity in the low reactive range compared to Alinity i and Maglumi X3.

Table 4: Clinical sensitivity results.

Assay	Analyzer	Cutoff	Unit	N	Negative	Positive	Mean	Sensitivity	Sensitivity 95%CI
AntiHCV	Alinity i	1	S/CO	100	0	100	9.85	100%	(96.30% / 100.00%)
	Maglumi X3	1	AU/mL	100	0	100	52.37	100%	(96.30% / 100.00%)
	CL1200i	1	COI	100	9	91	42.07	91%	(83.77% / 95.19%)
HBsAg	Alinity i	1	S/CO	152	0	152	66.76	100%	(97.54% / 100.00%)
	Maglumi X3	0.05	IU/mL	152	4	148	1.17	97.37%	(93.43% / 98.97%)
	CL1200i	0.05	IU/mL	152	23	129	0.07	84.87%	(78.32% / 89.70%)
HAV IgM	Alinity i	0.8	S/CO	100	0	100	7.38	100%	(96.30% / 100.00%)
	Maglumi X3	1	AU/mL	100	1	99	6.49	99%	(94.55% / 99.82%)
	CL1200i	1	COI	100	8	92	5.74	92%	(85.00% / 95.89%)

Clinical sensitivity evaluation results for HBsAg

Alinity i demonstrated the highest clinical sensitivity, correctly identifying all 152 positive samples (100%; 95% CI: 97.54-100.00%), with no false negative results observed. Maglumi X3 identified 148 of 152 positive samples, corresponding to a clinical sensitivity of 97.37% (95% CI: 93.43-98.97%), while CL-1200i correctly classified 129 of 152 positive samples, yielding a clinical sensitivity of 84.87% (95% CI: 78.32-89.70%) (see Table 4). A higher proportion of low signal samples near the cutoff was observed for Maglumi X3 and CL-1200i, consistent with the increased number of false negative results on these systems (Supplementary Figure 1B).

Evaluation using the HBsAg Low Titer Performance Panel and the HBsAg Sensitivity Panel further highlighted differences among the three systems (Table 5A). Alinity i correctly identified all panel samples, whereas Maglumi X3 failed to detect 3 low titer positive samples and CL-1200i identified correctly only 12 of the 20 low titer positive samples (Table 5B). In the HBsAg Sensitivity Panel 2410-0161, all HBV subtypes were detected by Alinity i and Maglumi X3, while one subtype (ay, panel member 14) was not detected by CL-1200i. Seroconversion panel testing demonstrated earlier HBV detection for Alinity i compared to the other systems (see Table 5A). Across the six seroconversion panels, Alinity i showed the shortest mean time to first HBsAg detection (10.7 days), followed by Maglumi X3 (21.2 days), while CL-1200i exhibited the longest mean detection time (28.5 days). In several panels, CL-1200i did not detect HBsAg within the observation period (Supplementary Figure 2).

Table 5: Days until detection and Low Titre Clinical Sensitivity for HBsAg.

No. Crt.	Seroconversion Panel ID	Alinity i	Maglumi X3	CL-1200i
1	0605-0041	0	11	14
2	0605-0039	0	9	13
3	0605-0038	0	5	7
4	0605-0059	2	18‡	>28 (ND)*
5	67303	62	72	79
6	65449	0	12	26
Mean number of days until detection		**10.7**	**21.2**	**28.5**
Number of positive results (out of total 104 test results)		**80 (76.9%)**	**52 (50%)**	**32 (30.7%)**

No. Crt.	Seroconversion Panel ID	Alinity i	Maglumi X3	CL-1200i
(B)				
1	0805-0417	20/20	17/20	12/20
2	2410-0161	15/15	15/15	14/15
Number of correctly identified results (out of total 70 duplicate test results)		**70 (100%)**	**64 (91.4%)**	**52 (74.3%)**

(A) Days until 1st HBsAg detection (Seroconversion Panels). No detection is denoted with ND. Asterisk (*) indicates that no signal was detected. Cross (‡) denotes that the Maglumi X3 device had intermittent detection (on days 18 and 28 – see Supplementary Figure 2)
(B) Clinical sensitivity evaluation for HBsAg determined using the Low Titre Performance Panel and the HBsAg Sensitivity Panel (Correctly Identified Samples).

Clinical sensitivity evaluation results for HAV IgM assay

Alinity i demonstrated a clinical sensitivity of 100%, correctly identifying all 100 positive samples, with no false negative results observed (see Table 4). Maglumi X3 correctly identified 99 of 100 positive samples, corresponding to a clinical sensitivity of 99.0% (95% CI: 94.55-99.82%), while CL-1200i identified 92 of 100 positive samples, yielding a clinical sensitivity of 92.0% (95% CI: 85.00-95.89%). Of the CLIA systems tested, CL-1200i exhibited a higher proportion of low signal results close to the cutoff, consistent with the increased number of false negative results compared to Alinity i and Maglumi X3 (Supplementary Figure 1C).

Clinical specificity evaluation results

Clinical specificity evaluation results for Anti-HCV

Clinical specificity results for the Anti-HCV assays (Table 6) reveal that from a total of 501 leftover patient samples tested, 19 samples were found reactive on at least one analyzer. One sample was confirmed positive by HCV RNA PCR testing (HCV viral load of 5,210 IU/mL) and was excluded from the specificity analysis, this sample was correctly identified as positive on Alinity i (mean result of 4.030 S/Co) and Maglumi X3 (mean result of 16.824 AU/mL), but non-reactive on CL-1200i.The remaining 18 samples were confirmed negative and were considered false reactive results. Clinical specificity calculations were therefore performed using the remaining 500 negative samples. Alinity i demonstrated the highest clinical specificity for the Anti-HCV assay, with a specificity of 99.80% (95% CI: 98.89 to 99.99%), corresponding to one false reactive result among the 500 negative samples. Maglumi X3 exhibited 98.80% (95% CI: 97.41 to 99.56%), with six false reactive results, while CL-1200i showed the lowest performance at 97.80% (95% CI: 96.10-98.90%), with eleven false reactive results.

Table 6: Clinical specificity.

Assay	Analyzer	Cutoff	Unit	N	Negative	Positive	Mean	Specificity	Sensitivity 95%CI
AntiHCV	Alinity i	1	S/CO	500	499	1	0.13	99.80%	(98.89% / 99.99%)
	Maglumi X3	1	AU/mL	500	494	6	0.36	98.80%	(97.41% / 99.56%)
	CL1200i	1	COI	500	489	11	0.20	97.80%	(96.10% / 98.90%)
HBsAg	Alinity i	1	S/CO	1500	1497	3	0.32	99.80%	(99.42% / 99.96%)
	Maglumi X3	0.05	IU/mL	1500	1498	2	0.03	99.87%	(99.52% / 99.98%)
	CL1200i	0.05	IU/mL	1500	1491	9	0.02	99.40%	(98.86% / 99.73%)
HAV IgM	Alinity i	0.8	S/CO	300	299	1	0.13	99.67%	(98.16% / 99.99%)
	Maglumi X3	1	AU/mL	300	298	2	0.47	99.33%	(97.61% / 99.92%)
	CL1200i	1	IU/mL	300	295	5	0.55	98.33%	(96.15% / 99.46%)

The vast majority of negative results (including the 50 samples from pregnant women) clustered well below the assay cutoffs across all three CLIA systems (Supplementary Figure 3A). The Alinity i demonstrated the greatest statistical separation between the negative sample distribution and the cutoff threshold (distance to cutoff: 12.33 standard deviations – SD), whereas the Maglumi X3 and CL-1200i exhibited narrower margins (1.15 SD for both). While false-reactive results were predominantly observed close to the assay cutoffs, isolated samples with higher reactive values were recorded on the CL-1200i and Maglumi X3.

Among the subgroup of 50 pregnant patients, one false-reactive result was observed on the Alinity i. This sample tested negative on both the Maglumi X3 and CL-1200i. The Maglumi X3 produced a false-reactive result from a different sample within this cohort. The CL-1200i produced zero false-reactive results among the pregnant patient samples.

Clinical specificity evaluation results for HBsAg

Clinical specificity results for the HBsAg assays (Table 6) reveal that from a total of 1,500 leftover patient samples tested, 13 samples were found reactive on at least one analyzer (one sample was false positive on both Alinity i and Maglumi X3). All reactive samples were confirmed negative by HBV DNA PCR testing and were considered false positive results. Alinity i demonstrated a clinical specificity of 99.80% (95% CI: 99.42 to 99.96%), with three false reactive results, while Snibe Maglumi X3 showed 99.87% (95% CI: 99.52 to 99.98%), corresponding to two false reactive results. Mindray CL-1200i exhibited 99.40% (95% CI: 98.86 to 99.73%), with 9 false reactive results among the 1,500 negative samples.

The majority of negative samples across all assays clustered well below their respective cutoffs (Supplementary Figure 3B). Reflecting the false-positive rates, the systems demonstrated distinct statistical separation between the negative sample distributions and their respective cutoff thresholds. The Alinity i showed the widest margin (distance to cutoff: 5.83 SD), followed by the Maglumi X3 (3.18 SD). In contrast, the CL-1200i exhibited a notably narrower margin (0.53 SD), which aligns with its higher incidence of false reactivity.

Clinical specificity evaluation results for HAV IgM assay

Clinical specificity results for the HAV IgM assays (Table 6) show that from a total of 300 samples tested, 6 were found reactive on at least one analyzer. All reactive samples were confirmed negative by HAV RNA PCR testing and were considered false positive results. One of the reactive samples was identified as positive on all three analyzers (mean result of 2.237 S/Co on Alinity i; 3.235 AU/mL on Maglumi X3; 5.130 COI on CL-1200i), however, HAV RNA was not detected, and no clinical evidence of acute HAV infection was available, therefore, this sample was classified as false reactive. Alinity i demonstrated a clinical specificity of 99.67% (95% CI: 98.16 to 99.99%), corresponding to one false positive result among the 300 negative samples. Maglumi X3 showed 99.33% (95% CI: 97.61 to 99.92%), with two false positive results, while CL-1200i exhibited 98.33% (95% CI: 96.15 to 99.46%), corresponding to five false positive results.

The majority of negative samples across all three systems clustered well below their respective assay cutoffs (Supplementary Figure 3C). Consistent with the observed false-reactive profiles, the systems displayed distinct degrees of statistical separation between their negative sample distributions and the cutoff thresholds. The Alinity i demonstrated the widest margin (distance to cutoff: 5.34 SD), followed by the Maglumi X3 (2.30 SD). In contrast, the CL-1200i exhibited the narrowest separation (0.68 SD), with its false-reactive results primarily occurring at low signal values just above this threshold.

Discussion

Our evaluation reveals that while all tested analytical systems are suitable for routine screening, their distinct performance profiles at the low reactive end have significant implications for clinical decision making and public health outcomes. These differences are most pronounced in the detection of nascent infections and low level antigenemia, suggesting that the selection of an analytical system must be guided by the specific diagnostic requirements of the patient population and the screening environment rather than operational metrics alone.

Analytical precision

All systems demonstrated acceptable analytical precision for routine diagnostic use. Alinity i consistently achieved precision values well below manufacturer claimed limits across all assays reflecting high within and total lab precision [28–30]. Maglumi X3 and CL-1200i also demonstrated acceptable precision performance although higher variability was observed, particularly for negative control materials near the cutoff values. The increased imprecision observed with the negative or near cutoff level controls across all systems does not affect clinical interpretation, as results remain below reactive thresholds. Importantly, the precision observed with positive control levels, which is clinically relevant for result confirmation and patient monitoring, was robust for all systems. Instances in which manufacturer claimed precision specifications were not fully met likely reflect the controlled manufacturer validation environments [31,32].

Analytical sensitivity and low level HBsAg detection

While the WHO guidance emphasizes the importance of using highly sensitive HBsAg assays, it does not prescribe a numeric requirement for analytical sensitivity [4]. However, we demonstrate that substantial differences exist among the three CLIA systems, particularly at low antigen concentrations near the assay cutoffs. The Alinity i HBsAg Next Qualitative assay demonstrated the the highest analytical sensitivity for low level antigen detection, followed by the Maglumi X3, while the CL 1200i showed a narrower range of reactivity. These findings

are broadly consistent with manufacturer reported analytical sensitivity claims [28,30].

From a clinical perspective, enhanced analytical sensitivity for HBsAg detection is particularly important for blood donor screening, early infection detection, and the identification of low level or occult HBV infections [9]. In these contexts, small analytical differences at low antigen concentrations may translate into delayed or even failed detection during early seroconversion stages or for samples with low level antigen concentration. This has important implications for transfusion safety and clinical management [34]. Furthermore, in high throughput screening settings, the differences in analytical sensitivity become crucial given that serological assays serve as the primary barrier prior to nucleic acid testing [4,5].

Endogenous interferences

None of the tested substances produced clinically significant interference across the three evaluated CLIA systems. Although statistically significant variations were observed under selected conditions, these effects did not alter qualitative result interpretation, as no sample crossed the assay cutoff. In accordance with CLSI EP07 guidance, interference effects were interpreted based on their potential impact on clinical decision rather than statistical significance alone [27]. For qualitative immunoassays, analytical signal variations exceeding predefined relative difference thresholds may be acceptable provided that reactive versus non-reactive classification remains unchanged [27,35]. In this study, all observed interference effects, including those exceeding ±10% relative difference, were therefore considered analytically but not clinically significant. Among the evaluated systems, Snibe Maglumi X3 demonstrated slightly greater signal variability in the presence of selected endogenous substances, particularly creatinine and lactate, compared with Alinity i and CL-1200i. However, these variations did not compromise result classification on any platform. Overall, the findings confirm adequate resistance to clinically relevant endogenous interference for all three systems, supporting their suitability for routine diagnostic and screening applications [27,35].

Clinical sensitivity and early seroconversion performance

Across all three viral hepatitis markers evaluated, Alinity i consistently demonstrated better clinical sensitivity, detecting all confirmed positive cases across the assay spectrum. Maglumi X3 provided a slightly lower, overall detection profile. In contrast, while performing within clinically acceptable margins for general routine screening, CL-1200i exhibited a pronounced reduction in identifying true positive samples. Notably, the false-negative results observed on the CL-1200i (and to a lesser extent on Maglumi X3) were predominantly associated with samples exhibiting low signal values near the assay cutoffs on the other systems. This suggests a limitation in detecting weakly reactive samples which is important for the identification of early-stage infections, low-titer presentations, and immunologically atypical patients.

While this analytical limitation is consistent across the assays, the clinical and public health consequences vary critically by the target antigen. For Anti-HCV, failure to detect weakly reactive samples creates a meaningful diagnostic gap. Missing these infections delays confirmatory RNA testing, treatment initiation, and linkage to care [4,36]. This is especially concerning in prenatal screening, where altered maternal antibody responses can occur, and undetected infection prevents appropriate follow-up, counselling, and postnatal monitoring of exposed infants [37].

For HBsAg, the risk of low-end sensitivity limitations is most vividly illustrated during early seroconversion. By substantially narrowing the diagnostic window, Alinity i detected nascent HBV infections considerably earlier and with greater frequency than the other systems. Conversely, CL-1200i missed a substantial proportion of low-titer samples and exhibited markedly delayed (or entirely absent) detection across full seroconversion series. This poses a direct threat to high-throughput blood donor screening, where identifying window-period infections is the primary barrier against onward transmission [8].

Lastly, for HAV IgM, because the clinical presentation of acute viral hepatitis is indistinguishable across different viral etiologies, rapid serological confirmation is paramount [11]. Missing low-level HAV IgM reactivity delays essential infection control measures and outbreak management. Furthermore, while HAV does not establish chronic infection, it can lead to fatal fulminant acute liver failure [38]. Relying on robust front-line serology is essential, as nucleic acid testing (while capable of identifying early infections) is often too costly and specialized for routine acute diagnosis [11,38].

Ultimately, these findings underscore that the clinical sensitivity of CLIA systems is a paramount consideration in assay selection, highlighting the clinical utility of a stratified, fit-for-purpose testing strategy [7]. Cost-effective systems that perform within acceptable clinical margins may be appropriately utilized for the routine monitoring of known positive patients, where analyte titers are typically well-established and the risk of missing a nascent infection is absent. Conversely, deploying systems with maximal analytical sensitivity (such as Alinity i) is imperative for initial screening in high-risk scenarios (blood donation, diagnostically complex patients, and early infection detection) where minimizing the diagnostic window period is critical [4].

In particular, highly sensitive screening in pregnant women is vital to detect low-level reactivity and ensure appropriate clinical interventions that prevent mother-to-child transmission [39]. While systems with maximal sensitivity, such as Alinity i, are optimal for preventing missed infections in this high-risk group, our evaluation revealed distinct specificity profiles within this demographic. In our cohort of pregnant patients, the CL-1200i platform demonstrated superior specificity for Anti-HCV screening by yielding zero false-reactive results. Conversely, the isolated false-reactive Anti-HCV results

observed on both the Alinity i and Maglumi X3 systems originated directly from this pregnant cohort, highlighting a well-documented clinical trade-off where ultra-high analytical sensitivity may occasionally capture pregnancy-induced immunological cross-reactivity. Therefore, the strategic deployment of highly sensitive screening assays is a fundamental requisite for advancing the WHO's goal of eliminating viral hepatitis as a public health threat by 2030 [1,2].

Clinical specificity and false-reactive

All evaluated systems demonstrated acceptable clinical specificity (>97.8%), confirming their overall diagnostic reliability for routine screening. However, distinct performance differences emerged among the systems. Alinity i and Maglumi X3 consistently exhibited the lowest false-positive rates across the evaluated markers. In contrast, while CL-1200i performed well within acceptable clinical parameters, it demonstrated a greater incidence of borderline reactivity and a comparatively higher proportion of false-reactive results, particularly in the Anti-HCV and HAV IgM assays.

These distinct performance profiles are directly explained by the assays' statistical separation between their negative sample distributions and their respective cutoff thresholds. Across all three viral markers, Alinity i maintained the widest statistical margin (measured as standard deviations – SD – to the cutoff), indicating that its baseline negative population clusters safely away from the reactive threshold. This robust margin makes the system highly resistant to minor biological noise and non-specific cross-reactivity. Conversely, the CL-1200i consistently exhibited the narrowest SD margins. When the baseline negative distribution sits in such close proximity to the cutoff, even minor assay signal fluctuations or low-level endogenous interference can push a true-negative sample over the threshold. This dynamic directly accounts for the higher incidence of borderline and false-reactive results observed on the Mindray platform. Lastly, Maglumi X3 maintained an intermediate separation that proved sufficient to sustain high specificity.

A significant difference regarding Anti-HCV testing in pregnant patients was observed. The false-reactive results identified by Alinity i and Maglumi X3 occurred within this specific cohort, likely reflecting the known immunological alterations characteristic of pregnancy [40]. Specifically, pregnancy induces the production of cross-reactive maternal alloantibodies directed against fetal antigens, as well as transient increases in heterophile antibodies. These circulating proteins can non-specifically bind to the assay's capture or detection reagents, generating spurious reactive signals despite the absence of true viral specific antibodies.

From an operational and public health perspective, the observed differences in false-positive rates, although small on a percentage basis, can have substantial practical implications for high-volume screening laboratories. While false-reactive results do not carry the immediate transmission risks of a missed infection, their downstream effects are costly and disruptive. Suboptimal specificity inevitably triggers a cascade of unnecessary confirmatory PCR testing, unwarranted patient anxiety, and unjustified blood donor deferrals [41,42]. Consequently, prioritizing high-specificity systems is essential for minimizing the clinical, financial, and operational burdens inherent to large-scale screening programs.

Study limitations and future perspectives

To our knowledge, this is the first study to provide a comprehensive, head-to-head performance comparison of the Abbott Alinity i, Snibe Maglumi X3, and Mindray CL-1200i CLIA systems across multiple viral hepatitis markers. This comparative evaluation fills a critical gap in the literature, as real-world assessments are essential to guide laboratory assay selection and characterize system behavior in a medical laboratory.

This study has several limitations. Because the evaluation was conducted retrospectively at a single center, the generalizability of the findings to other laboratory environments, workflows, or analyzer configurations may be limited. Furthermore, the assessment of viral genotype diversity was inherently restricted to the specific WHO reference materials and commercial panels available for inclusion. Long-term reagent stability and lot-to-lot variability were also not evaluated. Consequently, the results represent a snapshot of assay performance under the specific testing conditions, reagent lots, analyzer configurations, and software versions utilized during the study period. As such, performance characteristics may vary across different laboratories and over time.

Nevertheless, this foundational assessment establishes a robust baseline for these widely used screening systems. Future prospective studies incorporating multicenter evaluations, broader geographic datasets, and a wider array of clinical materials will be invaluable to further support, validate, and extend the findings of this comparative assessment.

Conclusion

Our study revealed significant performance disparities among the three CLIA systems. Based on the cumulative analytical and clinical data, this study establishes the comparative performance profiles for hepatitis serological screening: Alinity i demonstrated superior reliability over the Maglumi X3, which in turn outperformed the CL 1200i.

The Alinity i system provided the highest level of clinical sensitivity at 100% across all evaluated assays and achieved the most rapid detection of early HBV seroconversion. While the Maglumi X3 demonstrated high overall clinical sensitivity and specificity, it exhibited slightly lower sensitivity for low titer HBsAg samples and minor delays in seroconversion detection compared to the Alinity i. In contrast, the CL 1200i system exhibited a critical diagnostic defect, exhibiting a reduced clinical sensitivity of 84.87% for HBsAg and - delayed detection during early infection stages. These analytical

limitations directly translated into longer diagnostic window periods and a higher incidence of misclassified results compared to the other systems. For high throughput environments where early detection is the primary barrier to disease transmission, the use of analytical systems with limited low-end sensitivity poses a substantial risk to blood safety and public health. Consequently, laboratory selection must prioritize the categorical evidence of performance to ensure effective clinical intervention and actively support global hepatitis elimination targets.

Conflict of interest statement

D.G.C., S.D., and A.J.N. are affiliated to Dig Worldwide Ltd. Dig Worldwide Ltd was involved in the study design, data collection, analysis, and the decision to submit the manuscript for publication. D.G.C. is a contracted specialist working with Dig Worldwide Ltd, S.D. is an employee of, and A.J.N. is a shareholder of Dig Worldwide Ltd. Abbott Laboratories had visibility towards the data and the manuscript before submission without altering the results or their interpretation. The final publication decision remained independently with the authors. The authors declare no other competing interests.

Funding statement

This study was supported by Dig Worldwide Ltd., a commercial consultancy firm in the life sciences sector, utilizing project funding from Abbott Laboratories. This research was an initiative of Dig Worldwide Ltd. and the results presented in this study were not influenced or biased in any way by CLIA system manufacturers or other second or third parties.

Data availability statement

The analyzed data that support the findings of this study are available on request from the corresponding author, by emailing: dgc@dig-worldwide.com.

CRediT authorship contribution statement

Author Contributions: Conceptualization, D.C., S.D., A.N.; validation D.C., S.D., A.N.; investigation D.C., formal analysis S.D., D.C., A.N.; data curation D.C.; writing - original draft D.C.; writing - review & editing D.C., S.D., A.N.; visualization D.C., project administration D.C., S.D., A.N.; supervision A.N.; funding acquisition A.N.;

References

1. World Health Organization. Global health sector strategies on HIV, viral hepatitis and sexually transmitted infections for the period 2022-2030 https://www.who.int/publications/i/item/9789240053779 (accessed April 2, 2026).
2. CDC Reports Latest National Data on Syphilis in Newborns and Sexually Transmitted Infections (STIs) | CDC Newsroom n.d. https://www.cdc.gov/media/releases/2025/2025-cdc-reports-latest-national-data-on-syphilis-in-newborns-and-sexually-transmitted-infections-stis.html (accessed April 2, 2026).
3. World Health Organization. Global health sector strategies on, respectively, HIV, viral hepatitis and sexually transmitted infections for the period 2022-2030. GHSS 2022-2030 2022.
4. UNAIDS 2025 Global AIDS Update — AIDS, Crisis and the Power to Transform | UNAIDS n.d. https://www.unaids.org/en/resources/documents/2025/2025-global-aids-update-summary (accessed April 2, 2026).
5. Ghanem KG, Ram S, Rice PA. The Modern Epidemic of Syphilis. New England Journal of Medicine 2020;382:845-854. https://doi.org/10.1056/NEJMRA1901593;WGROUP:STRING:MMS.
6. WHO. Global Health Sector Strategies on HIV, Viral Hepatitis and the sexually transmitted infections. World Health Organization 2021;ector stra:2022-2230.
7. Kamb ML, Newman LM, Riley PL, Mark J, Hawkes SJ, Malik T, et al. A Road Map for the Global Elimination of Congenital Syphilis. Obstet Gynecol Int 2010;2010:312798. https://doi.org/10.1155/2010/312798.
8. U.S. Syphilis Cases in Newborns Continue to Increase: A 10-Times Increase Over a Decade | CDC Online Newsroom | CDC n.d. https://www.cdc.gov/media/releases/2023/s1107-newborn-syphilis.html (accessed April 2, 2026).
9. CDC Reports Latest National Data on Syphilis in Newborns and Sexually Transmitted Infections (STIs) | CDC Newsroom n.d. https://www.cdc.gov/media/releases/2025/2025-cdc-reports-latest-national-data-on-syphilis-in-newborns-and-sexually-transmitted-infections-stis.html (accessed April 2, 2026).
10. Moschese D, Lazzarin S, Colombo ML, Caruso F, Giacomelli A, Antinori S, et al. Breakthrough Acute HIV Infections among Pre-Exposure Prophylaxis Users with High Adherence: A Narrative Review. Viruses 2024, Vol 16, 2024;16. https://doi.org/10.3390/V16060951.
11. Seed CR, Styles CE, Hoad VC, Yang H, Thomas MJ, Gosbell IB. Effect of HIV pre-exposure prophylaxis (PrEP) on detection of early infection and its impact on the appropriate post-PrEP deferral period. Vox Sang 2021;116:379-387. https://doi.org/10.1111/VOX.13011.
12. Rodgers MA, Vallari AS, Yamaguchi J, Holzmayer V, Harris B, Toure-Kane C, et al. ARCHITECT HIV Combo Ag/Ab and RealTime HIV-1 Assays Detect Diverse HIV Strains in Clinical Specimens. AIDS Res Hum Retroviruses 2018;34:314. https://doi.org/10.1089/AID.2017.0244.
13. Miedouge M, Grèze M, Bailly A, Izopet J. Analytical sensitivity of four HIV combined antigen/antibody assays using the p24 WHO standard. Journal of Clinical Virology 2011;50:57–60. https://doi.org/10.1016/j.jcv.2010.09.003.
14. Association WM. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. JAMA 2013;310:2191-2194. https://doi.org/10.1001/JAMA.2013.281053.

15. International Organization for Standardization (ISO). ISO 15189:2022. Medical laboratories – requirements for quality and competence 2022.
16. Abbott Laboratories Abbott Ireland Diagnostics Division. Alinity i HIV Ag/Ab Combo Reagent Kit, Ref. No. 08P07, Instructions for Use [Package Insert]
17. Mindray Bio-Medical Electronics Co. Ltd. CL-series HIV Antigen and Antibodies to Human Immunodeficiency Virus (CLIA), Ref. No. HIV121, Instructions for Use [Package Insert]
18. Shenzhen New Industries Biomedical Engineering Co. Ltd. MAGLUMI® HIV Ab/Ag Combi (CLIA), Ref. No. 130219008M, Instructions for Use [Package Insert]
19. NIBSC (National Institute for Biological Standards and Control), WHO International Laboratory for Biological Standards, UK Official Medicines Control Laboratory. International Reference Reagent, HIV-1 P24 Antigen, NIBS code: 90/636, Instructions for Use [Package Insert Version 6.0, Dated 12/12/2023].
20. Miedouge M, Grèze M, Bailly A, Izopet J. Analytical sensitivity of four HIV combined antigen/antibody assays using the p24 WHO standard. Journal of Clinical Virology 2011;50:57–60. https://doi.org/10.1016/j.jcv.2010.09.003.
21. Abbott Laboratories Abbott Ireland Diagnostics Division. Alinity i Syphilis Reagent Kit, Ref. No. 07P60, Instructions for Use [Package Insert]
22. Shenzhen New Industries Biomedical Engineering Co. Ltd. MAGLUMI® Syphilis (CLIA), Ref. No. 130269003M, Instructions for Use [Package Insert]
23. Mindray Bio-Medical Electronics Co. Ltd. CL-series Anti-TP, Antibody to Treponema Pallidum (CLIA), Ref. No. 105-005671-00, Instructions for Use [Package Insert]
24. Clinical and Laboratory Standards Institute. CLSI EP15-A3. User Verification of Precision and Estimation of Bias; Approved Guideline - 3rd Edition 2014.
25. NIBSC (National Institute for Biological Standards and Control), WHO International Laboratory for Biological Standards, UK Official Medicines Control Laboratory. WHO Reference Panel, 1st WHO International Reference Panel for HIV-1 p24 Antigen, NIBS code: 16/210, Instructions for Use [Package Insert Version 3.0, Dated 23/09/2024]
26. World Health Organization. WHO Expert Committee on Biological Standardization - Sixty-ninth report, Section 7.1.2 First WHO International Reference Panel for HIV-1 p24 antigen and First WHO International Reference Reagent for HIV-2 p26 antigen. WHO Technical Report Series, No 1016, 2019
27. LLC SD. ASSURANCETM Interference Test Kit INT-06 Instructions for Use [Package Insert]; Sample Interference Testing procedure (based on CLSI EP-7A guidelines), dilution calculator and results reporting spreadsheet
28. SeraCare LGC Group (LGC Limited. Guildford. UK). AccuSetTM HIV-1 Worldwide Performance Panel, Ref. 0800-0492, Instructions for use [Package Insert, Data Sheets] https://www.seracare.com/AccuSet-HIV-Worldwide-Performance-Panel-0800-0492/ 2021.
29. SeraCare LGC Group (LGC Limited. Guildford. UK). AccuVertTM HIV-1 Seroconversion Panel, Ref. 0600-0243, Instructions for use [Package Insert, Data Sheets] https://www.seracare.com/AccuVert-HIV1-Seroconversion-Panel-06000243-0600-0243/ 2023.
30. SeraCare LGC Group (LGC Limited. Guildford. UK). AccuVertTM HIV-1 Seroconversion Panel, Ref. 0600-0245, Instructions for use [Package Insert, Data Sheets] https://www.seracare.com/AccuVert-HIV1-Seroconversion-Panel-06000245-0600-0245/ 2023.
31. SeraCare LGC Group (LGC Limited. Guildford. UK). AccuVertTM HIV-1 Seroconversion Panel, Ref. 0600-0248, Instructions for use [Package Insert, Data Sheets] https://www.seracare.com/AccuVert-HIV1-Seroconversion-Panel-06000248-0600-0248/ 2022.
32. SeraCare LGC Group (LGC Limited. Guildford. UK). AccuVertTM HIV-1 Seroconversion Panel, Ref. 0600-0252, Instructions for use [Package Insert, Data Sheets] https://www.seracare.com/AccuVert-HIV1-Seroconversion-Panel-06000252-0600-0252/ 2022.
33. SeraCare LGC Group (LGC Limited. Guildford. UK). AccuVertTM HIV-1 Seroconversion Panel, Ref. 0600-0258, Instructions for use [Package Insert, Data Sheets] https://www.seracare.com/AccuVert-HIV1-Seroconversion-Panel-06000258-0600-0258/ 2024.
34. SeraCare LGC Group (LGC Limited. Guildford. UK). AccuVertTM HIV-1 Seroconversion Panel, Ref. 0600-0260, Instructions for use [Package Insert, Data Sheets] https://www.seracare.com/AccuVert-HIV1-Seroconversion-Panel-06000260-0600-0260/ 2023.
35. SeraCare LGC Group (LGC Limited. Guildford. UK). AccuVertTM HIV-1 Seroconversion Panel, Ref. 0600-0262, Instructions for use [Package Insert, Data Sheets] https://www.seracare.com/AccuVert-HIV1-Seroconversion-Panel-06000262-0600-0262/ 2023.
36. SeraCare LGC Group (LGC Limited. Guildford. UK). AccuVertTM HIV-1 Seroconversion Panel, Ref. 0600-0265, Instructions for use [Package Insert, Data Sheets] https://www.seracare.com/AccuVert-HIV1-Seroconversion-Panel-06000265-0600-0265/ 2021.
37. SeraCare LGC Group (LGC Limited. Guildford. UK). AccuVertTM HIV-1 Seroconversion Panel, Ref. 0600-0271, Instructions for Use [Package Insert, Data Sheets] https://www.seracare.com/AccuVert-HIV1-Seroconversion-Panel-06000271-0600-0271/ 2024.
38. SeraCare LGC Group (LGC Limited. Guildford. UK). AccuVertTM HIV-1 Seroconversion Panel, Ref. 0600-0272, Instructions for use [Package Insert, Data Sheets] https://www.seracare.com/AccuVert-HIV1-Seroconversion-Panel-06000272-0600-0272/ 2022.
39. Fiebig EW, Wright DJ, Rawal BD, Garrett PE, Schumacher

RT, Peddada L, et al. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. AIDS 2003;17:1871-1879. https://doi.org/10.1097/00002030-200309050-00005.
40. Pandori MW, Hackett J, Louie B, Vallari A, Dowling T, Liska S, et al. Assessment of the Ability of a Fourth-Generation Immunoassay for Human Immunodeficiency Virus (HIV) Antibody and p24 Antigen To Detect both Acute and Recent HIV Infections in a High-Risk Setting. J Clin Microbiol 2009;47:2639. https://doi.org/10.1128/JCM.00119-09.
41. Chéret A. Acute HIV-1 Infection: Paradigm and Singularity. Viruses 2025;17:366. https://doi.org/10.3390/v17030366.
42. Gaspari V, Filippini A, Orioni G, Mussi M, Carpanese MA, La Placa M, et al. The Rise of Syphilis Infections and Reinfections over a Decade (2009–2019) in the Bolognese Area: A Retrospective Analysis. Microorganisms 2025;13:285. https://doi.org/10.3390/microorganisms13020285.
43. Nair M, Gettins L, Fuller M, Kirtley S, Hemelaar J. Global and regional genetic diversity of HIV-1 in 2010–21: systematic review and analysis of prevalence. Lancet Microbe 2024;5. https://doi.org/10.1016/S2666-5247(24)00151-4.
44. Regulation - 2017/746 - EN - Medical Device Regulation - EUR-Lex n.d. https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32017R0746 (accessed April 2, 2026).
45. World Health Organization (Geneva). World Health Organization (2024). Consolidated guidelines on differentiated HIV testing services n.d.
46. Totten YR, Hardy BM, Bennett B, Rowlinson MC, Crowe S. Comparative Performance of the Reverse Algorithm Using Architect Syphilis TP Versus the Traditional Algorithm Using Rapid Plasma Reagin in Florida's Public Health Testing Population. Ann Lab Med 2019;39:396-399. https://doi.org/10.3343/ALM.2019.39.4.396.
47. Morshed MG, Singhb AE. Recent trends in the serologic diagnosis of syphilis. Clin Vaccine Immunol 2015;22:137-143. https://doi.org/10.1128/CVI.00681-14.

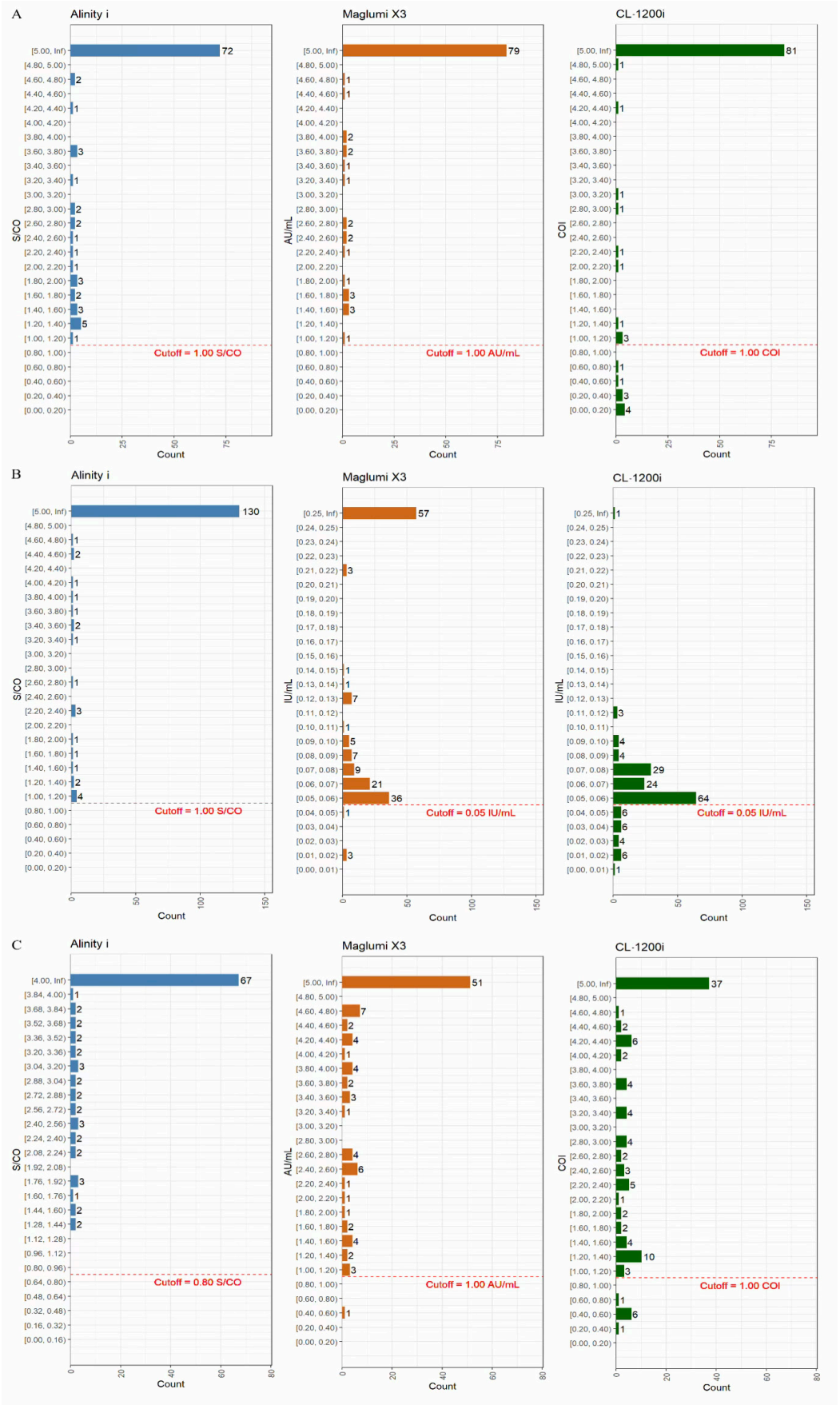
Supplementary Table 1: Characteristics and technical specifications of each system.

	Alinity i	Maglumi X3	CL1200i
Manufacturer	Abbott Laboratories Inc.	Shenzhen New Industries Biomedical Engineering (Snibe) Co., Ltd.	Mindray BioMedical Electronics Co., Ltd.
Analyzer model	Alinity i	Maglumi X3	CL1200i
Method	Chemiluminescent microparticle immunoassay (CMIA)		
Measurement principle	Flash CLIA	Flash CLIA	Glow CLIA
CLIA Label	Acridinium	ABEI (Isoluminol derivate)	ALP
CLIA Substrate	NaOH, H2O2	NaOH, H2O2	AMPPD
Solid phase	paramagnetic microparticles	paramagnetic nanoparticles	paramagnetic microparticles
Capacity	200 tests/hour	200 tests/hour	180 tests/hour

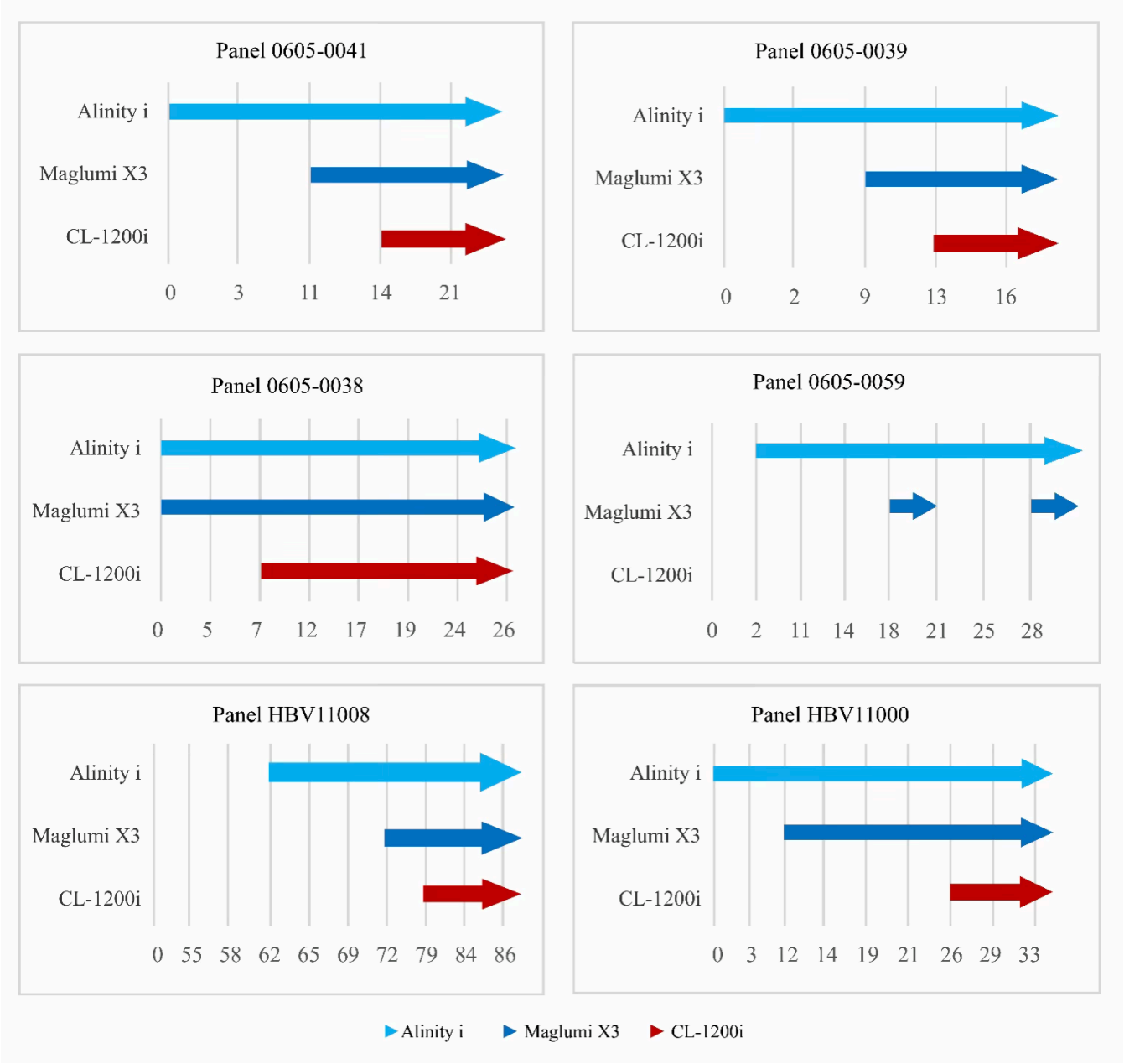
Supplementary Table 2: Precision evaluation results for Anti-HCV, HBsAg and HAV IgM.

Assay	Material	Analyzer	N	Mean	Unit	Repeatability		Within-Lab		95% Lower CL Within-Lab		95% Upper CL Within-Lab	
						(SD)	(CV%)	(SD)	(CV%)	(SD)	(CV%)	(SD)	(CV%)
Anti-HCV	Negative Control (-)	Alinity i	25	0.08	S/CO	0.004	5.35	0.006	7.06	0.004	5.06	0.010	11.66
		Maglumi X3	25	0.31	AU/mL	0.016	5.12	0.165	53.39	0.099	32.07	0.469	151.7
		CL-1200i	25	0.13	COI	0.012	9.38	0.013	9.92	0.010	7.66	0.018	14.07
	Positive Control (+)	Alinity i	25	4.16	S/CO	0.062	1.48	0.066	1.59	0.051	1.22	0.094	2.27
		Maglumi X3	25	9.82	AU/mL	0.382	3.89	0.788	8.02	0.508	5.17	1.736	17.67
		CL-1200i	25	3.28	COI	0.158	4.82	0.158	4.82	0.123	3.76	0.22	6.71
HBsAg	Negative Control (-)	Alinity i	25	0.32	S/CO	0.023	7.08	0.027	8.39	0.020	6.25	0.041	12.77
		Maglumi X3	25	0.03	IU/mL	0.004	14.69	0.005	16.19	0.004	12.37	0.007	23.47
		CL-1200i	25	0.02	IU/mL	0.002	10.70	0.005	28.18	0.003	17.65	0.011	68.31
	Positive Control (+)	Alinity i	25	3.26	S/CO	0.068	2.07	0.068	2.07	0.053	1.61	0.094	2.88
		Maglumi X3	25	1.02	IU/mL	0.036	3.54	0.036	3.58	0.028	2.79	0.051	4.99
		CL-1200i	25	0.57	IU/mL	0.042	7.49	0.042	7.49	0.033	5.84	0.059	10.43
	Positive Control (2)	Maglumi X3	25	100.9	IU/mL	3.258	3.23	4.437	4.40	3.147	3.12	7.512	7.45
HAV IgM	Negative Control (-)	Alinity i	25	0.14	S/CO	0.009	6.80	0.011	8.08	0.008	6.01	0.017	12.34
		Maglumi X3	25	0.54	AU/mL	0.044	8.08	0.045	8.29	0.035	6.44	0.063	11.61
		CL-1200i	25	0.48	COI	0.020	4.17	0.021	4.33	0.016	3.36	0.029	6.09
	Positive Control (+)	Alinity i	25	1.81	S/CO	0.061	3.39	0.062	3.43	0.048	2.67	0.087	4.78
		Maglumi X3	25	4.4	AU/mL	0.172	3.90	0.172	3.90	0.134	3.04	0.239	5.43
		CL-1200i	25	2.92	COI	0.133	4.55	0.133	4.55	0.103	3.55	0.185	6.33

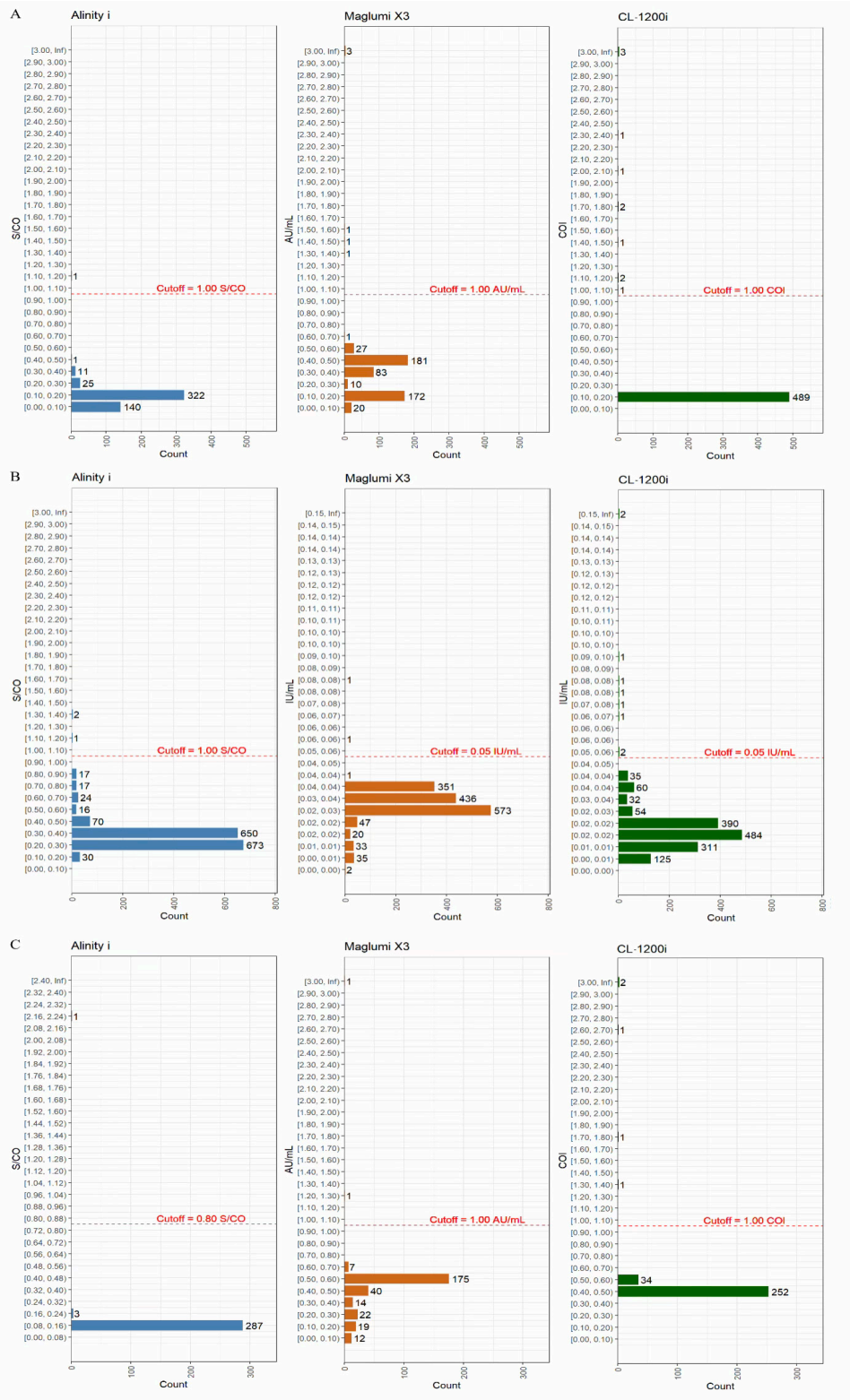
Supplementary Figure 1: Clinical sensitivity evaluation results distribution on the three CLIA platforms. (A) Anti-HCV; (B) HBsAg (C) HAV IgM.



Supplementary Figure 2: Number of days since the 1st HBsAg positive test result detection. Colour coded arrows are shown for each of the analyzers (panel members tested negative between first and last positive detection, are presented by separate arrows, indicating the intermittent detection). Arrows heads exceed the last detection days to reflect that the sample will be detected as positive from then onwards.



Supplementary Figure 3: Clinical specificity evaluation results distribution on the three CLIA platforms. (A) Anti-HCV; (B) HBsAg (C) HAV IgM.



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