Research Article

Evaluation of Infectious Disease Immunoassays for HIV 1/2 Ab-Ag Combo and Syphilis Ab on Alinity i, Maglumi X3 and CL-1200i CLIA Systems

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Abstract

Background: To support global HIV and syphilis elimination, highly sensitive serological screening is essential. Early detection is critical, as evolving diagnostic landscapes can alter antigen-antibody dynamics. Independent evaluations are necessary to assess the real-world performance of 4th generation assays in detecting low-level biomarkers during the seroconversion window.

Objectives: Performance evaluation and comparison of HIV and syphilis infectious disease serology screening across multiple analytical performance studies conducted on three commercially available automated chemiluminescent immunoassay (CLIA) systems.

Materials & Methods: Performance of the HIV 1/2 Ab-Ag Combo and Syphilis immunoassays was evaluated across three CLIA systems: Abbott Alinity i (Abbott Laboratories Inc., Abbott Park, Illinois, USA), Snibe Maglumi X3 (Shenzhen New Industries Biomedical Engineering Co., Ltd., Shenzhen, China), and Mindray CL-1200i (Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China). HIV analytical sensitivity was assessed using the 1st WHO International Reference Panel (NIBSC 16/210), representing 10 diverse subtypes and Group O. Clinical performance was determined using 132 specimens from 10 seroconversion panels, 15 HIV subtype samples, and 1,500 negative patient samples. For syphilis, clinical sensitivity and specificity were established using 250 confirmed positive and 250 negative clinical specimens. All testing followed ISO 15189:2022 and CLSI guidelines.

Results: All systems demonstrated acceptable analytical precision for routine clinical use. For the HIV reference panel, the Alinity i consistently exhibited the highest analytical sensitivity across all 10 subtypes and Group O (mean: 0.728 IU/mL; range: 0.658-0.764 IU/mL), followed by Maglumi X3 (mean: 0.819 IU/mL; range: 0.790-0.880 IU/mL) and CL-1200i (mean: 2.313 IU/mL; range: 2.05-2.60 IU/mL). These analytical differences corresponded to distinct variations in early detection windows across the

evaluated seroconversion panels; the mean time until the first reactive result was 11.8 days (median: 9.0; range: 0-35) for Alinity i (59.1% overall detection), 15.8 days (median: 13.5; range: 0-37) for Maglumi X3 (46.9% detection), and 19.1 days (median: 16.0; range: 7-44) for CL-1200i (34.8% detection). Clinical specificity for HIV was 99.87% (1498/1500) for Alinity i, while both Maglumi X3 and CL-1200i demonstrated a higher number of false reactive results (n=5 for both vs. n=2 for Alinity i), resulting in a lower specificity of 99.67% (1495/1500). For syphilis, clinical sensitivity was 99.60% for Alinity i and 99.20% for Maglumi X3, whereas CL-1200i showed a diagnostic gap at 96.00%. Syphilis specificity was 99.20% for Alinity i and Maglumi X3, compared to 97.20% for CL-1200i.

Conclusions: Per the In Vitro Diagnostic Regulation (IVDR), post-market evaluations of infectious disease immunoassays are increasingly critical. Our findings underscore this necessity by revealing significant analytical and clinical performance differences among widely used CLIA systems. These variations carry direct implications for the reliable detection of early infections, and suggest important hypotheses regarding the potential operational impact on the diagnosis and monitoring of individuals receiving PrEP and early ART. In these increasingly common clinical scenarios, even minor analytical differences between assays represent potential diagnostic gaps that warrant careful consideration within real-world testing workflows.

Introduction

Human immunodeficiency virus (HIV) and syphilis remain significant global public health concerns, posing ongoing risks to blood safety and maternal-fetal health [1–4]. HIV targets the immune system, progressively weakening host defences against opportunistic infections and certain cancers. The global burden of HIV remains staggering; by the end of 2024, approximately 40.8 million people were living with the virus, with an estimated 1.3 million new infections occurring that year [1]. The virus is primarily transmitted through the exchange of bodily fluids via sexual contact, unsafe blood transfusions, and from mother to child. Syphilis is a curable sexually transmitted infection (caused by the bacterium Treponema pallidum) that is experiencing a global resurgence [1,5]. Recent data indicates that approximately 8 million adults acquire syphilis annually [1,2,6]. Like HIV, syphilis is transmitted through sexual contact, unsafe blood practices, and during pregnancy. The impact of maternal syphilis is particularly devastating [7], resulting in over hundreds of thousands of congenital syphilis cases worldwide [8], causing significant adverse birth outcomes globally [9]. Furthermore, syphilis infection is consistently associated with an increased risk of HIV acquisition. Despite the availability of effective prevention and treatment strategies [9], both infections persist across diverse populations, including blood donors, pregnant women, and, although rare, individuals intermittently utilizing pre-exposure prophylaxis (PrEP) [2,10]. Therefore, highly accurate and reliable laboratory screening remains essential for timely diagnosis and public health prevention and control.

Serological testing represents the primary entry point for diagnosing HIV and syphilis in most screening algorithms. However, early infections are frequently asymptomatic and may feature low or delayed antibody responses. For HIV, this diagnostic challenge is increasingly relevant in the context of expanded PrEP utilization and early antiretroviral therapy (ART) initiation [11]. While these interventions are critical for patient care, exposure to antiretrovirals can significantly suppress or alter typical serological dynamics, potentially causing false-negative or borderline results during acute infection. Furthermore, the combination of these blunted antibody responses and the broad diversity of circulating HIV subtypes necessitate highly sensitive assays capable of robust performance across complex clinical profiles [12]. Ultimately, diagnostic delays caused by assay limitations can postpone confirmatory nucleic acid testing, treatment optimization, and critical partner notification.

Since syphilis cannot be reliably distinguished from other conditions on clinical grounds alone, laboratory confirmation is vital [11]. Test interpretation can be complicated by the infection stage, the host immune response, and physiological states such as pregnancy. Untreated syphilis can last for years and cause severe health issues. Missed or delayed detection is particularly dangerous for pregnant women; inadequate or delayed treatment can result in congenital infection in 50% to 80% of cases, leading to adverse outcomes such as stillbirth, neonatal death, prematurity, and low birth weight [11].

Automated chemiluminescent immunoassay (CLIA) systems are widely used for high-throughput screening. However, differences in assay design and signal detection can significantly affect their analytical and clinical performance [13]. Therefore, evaluations under routine laboratory conditions are necessary to characterize real-world performance beyond manufacturer claims.

To address these diagnostic challenges, our study presents a comparative performance evaluation of HIV 1/2 Ab-Ag Combo and Syphilis immunoassays across three widely used automated CLIA platforms: the Abbott Alinity i, Snibe Maglumi X3 and Mindray CL-1200i. Conducted under routine clinical laboratory conditions, our assessment aims to characterize the real-world analytical and clinical performance of these CLIA devices beyond manufacturer claims. Ultimately, our study seeks to reveal if any practical differences in assay capabilities are present, thus providing evidence-based insights which can guide system selection for reliable screening.

Materials and Methods

Study design

This study was conducted under routine clinical laboratory conditions to reflect daily diagnostic conditions rather than controlled manufacturer validation environments. The study

protocol, objectives and the materials used are summarized in Table 1. All measurements were performed by trained laboratory personnel using standard operating procedures, routine calibration and quality control practices, and manufacturer recommended assay settings. 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 in Bucharest, Romania, and all procedures used fully de-identified residual serum samples collected after the completion of routine diagnostic testing. Under the institutional and national regulatory standards, 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, in accordance with the Declaration of Helsinki [14]. All procedures followed laboratory quality assurance guidelines set by the international standard ISO 15189:2022 [15], by the Clinical and Laboratory Standards Institute (CLSI) guidelines, including EP15-A3 (User Verification of Precision and Estimation of Bias) and EP07 (Interference Testing in Clinical Chemistry), under routine clinical laboratory conditions.

Table 1: The study protocol and materials used.

Study objectives					
Assay	**Precision**	**Analytical Sensitivity**	**Endogenous Interferents**	**Clinical Sensitivity**	**Clinical Specificity**
HIV 1/2 Combo	5 days (5 reps x 5 days) / Internal quality controls	1st WHO IRP HIV p24 Antigen panel (NIBSC 16/210)	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)	10 seroconversion panels and 15 subtype samples	1500 negative samples
Syphilis	5 days (5 reps x 5 days) / Internal quality controls	N/A		250 positives confirmed by RPR/VDRL/TPHA	250 negative samples

Abbreviations: WHO (World Health Organization); IRP (International Reference Panel); NIBSC (National Institute for Biological Standards and Control); N/A (Not applicable); RPR (Rapid Plasma Reagin); VDRL (Venereal Disease Research Laboratory); TPHA (Treponema Pallidum Hemagglutination Assay).

CLIA systems and immunoassays

Performance evaluations of the HIV 1/2 Ab-Ag Combo and Syphilis immunoassays (specific for each instrument) were conducted using three automated CLIA systems: the Abbott Alinity i (Abbott Laboratories Inc., Abbott Park, Illinois, USA), Snibe Maglumi X3 (Shenzhen New Industries Biomedical Engineering Co., Ltd., Shenzhen, China), and Mindray CL-1200i (Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China). While all three systems employ chemiluminescent detection principles, they differ in their luminescent labels, light emission kinetics, and technical specifications (Supplementary Table 1).

The evaluated HIV assays [16–18] are 4th generation chemiluminescent immunoassays designed for the simultaneous, qualitative detection of the HIV p24 antigen and antibodies to HIV-1 (group M and group O) and HIV-2. These assays serve as screening aids for HIV diagnosis and do not distinguish between antigen and antibody reactivity. All three device-specific assays share a similar analytical architecture: magnetic microparticles coated with HIV-1/HIV-2 antigens and anti-p24 antibodies capture the targets from the serum or plasma sample. Following a wash step, a labelled conjugate containing recombinant HIV-1/HIV-2 antigens and monoclonal anti-p24 antibodies is added. Subsequent washing and the addition of chemiluminescence catalysts generate a relative light unit (RLU) signal. This signal is directly proportional to the analyte concentration and is evaluated against a calibrated cutoff. The primary differences among the HIV assays stem from their specific conjugate labels, catalysts, and method traceability. The Maglumi X3 and CL-1200i assays are standardized against the 1st WHO International Reference Reagent for HIV-1 p24 Antigen (NIBSC code 90/636) [19]. Conversely, the Alinity i assay is referenced to the AFSSAPS international standard panel to maintain a consistent HIV-1 p24 antigen sensitivity of <50 pg/mL. These AFSSAPS standards were historically used to set lower limits of detection before widespread harmonization with the WHO international standard (IU/mL) [20]. Currently, no internationally accepted standards exist for anti-HIV 1 and anti-HIV 2 antibodies.

The evaluated syphilis assays [21–23] are qualitative CLIAs designed to detect total antibodies against Treponema pallidum (T. pallidum) as an aid in diagnosis. All three systems capture sample antibodies using microparticles coated with recombinant T. pallidum antigens, but they utilize different labelled conjugates to form the final sandwich complexes. The Alinity i and CL-1200i assays employ labelled anti-human IgM and IgG conjugates, whereas the Maglumi X3 utilizes a T. pallidum recombinant antigen conjugate. Like the HIV assays, the final RLU signal is proportional to the antibody concentration and compared to a calibrated cutoff. Because there are no international standards for syphilis testing, all three assays are referenced to distinct, manufacturer specific internal

standards, which contributes to the variations in their overall design and performance.

Analytical precision

Precision evaluation was conducted for all of the assays using the internal quality controls provided by each of the respective assay manufacturers, by performing 5 days of consecutive measurements, with 5 replicates in a single run on each day, with a total of 25 replicates for each quality control material. This protocol is consistent with the experimental design for precision verification as described in the Clinical and Laboratory Standards Institute (CLSI) EP15-A3 [24] guidelines (1 run x 5 reps x 5 days = 25 reps per level). The results for the precision study were compared between analyzers and relative to their respective manufacturer claimed performance.

Analytical sensitivity

Analytical sensitivity was evaluated for HIV 1/2 Combo Antigen-Antibody assays using the World Health Organization (WHO) 1st WHO Reference Panel for HIV-1 p24 Antigen (NIBSC code: 16/210) [25]. Panel members representing subtypes A1, B, C, D, G, H, circulating recombinant forms (CRFs): F1/CRF12_BF/BFrec, CRF20_BG, CRF01_AE, CRF02_AG, and group O, are provided lyophilized in individual vials. Panel member samples were reconstituted using 1 mL nuclease-free ultrapure water per vial on the day of testing and further used to prepare serial dilutions of the panel members. Unitage (IU/mL) values for the diluted samples were calculated according to the applied dilution factor, using the unitage values provided for the concentrated panel members. Although the WHO Expert Committee has not formally assigned a potency unitage to any of the panel members [26] the calibrated values relative to the 1st International Reference Reagent (NIBSC 90/636) are provided in International Units (IU) per 1 mL (IU/mL) for each panel member. Same day measurements were performed on all three analyzers by measuring 5 replicates for each dilution sample. Dilutions were further prepared and tested until two out of the three evaluated assays stopped detecting the tested dilution sample as reactive, returning negative results for all measurement replicates. Analytical sensitivity in this study was reported as a comparative performance characteristic and does not represent a formal limit of detection (LoD) determination. Because each dilution level was evaluated using 5 replicates rather than the larger replicate sizes required for statistical modeling, a formal probit analysis to calculate a 95% LoD was not feasible (see Statistical Analysis, below). Consequently, the reported sensitivity values should be interpreted strictly as comparative indicators of relative platform detection thresholds under identical experimental conditions, rather than absolute, established platform LoDs.

Endogenous interferences

Analytical interference for each assay was evaluated with seven common endogenous metabolites expected in a patient population using the ASSURANCE™ Interference Test Kit INT-06 (Sun Diagnostics, LLC, New Gloucester, Maine, USA) [27]. 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. The required amount of interferent materials were calculated using the Assurance™ Excel Worksheets [27]. For each test pool, a matching control pool was prepared with the same volume of solvent (purified water) as used in preparation of the corresponding interferent pool. 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 clinical 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 differently for each assay (Table 1). For HIV 1/2 Combo assays, evaluation was conducted using one subtype performance panel (15 panel members of plasma samples collected worldwide from individual donors positive for HIV, consisting of 8 distinct HIV genotypes, including several circulating recombinant forms and mixed subtypes. Represented in this panel are B, BF, BG, CPX, C, CRF01_AE, CRF02_AG, and F1 subtypes) and 10 seroconversion panels, using the AccuSet HIV-1 Worldwide Performance Panel 0800-0492 [28] and the AccuVert HIV-1 Seroconversion Panels 0600-0243 [29]; 0600-0245 [30]; 0600-0248 [31]; 0600-0252 [32]; 0600-0258 [33]; 0600-0260 [34]; 0600-0262 [35]; 0600-0265 [36]; 0600-0271 [37]; 0600-0272 [38] (SeraCare, LGC Group, LGC Limited, Guildford, UK). Clinical sensitivity evaluation for syphilis was conducted using 250 leftover positive patient samples, confirmed positive by RPR/VDRL/TPHA testing. Because these specimens consisted of fully de-identified residual sera obtained after routine

diagnostic testing, matching clinical records regarding disease stage (primary, secondary, or latent), historical treatment status, or exact non-treponemal titers were completely inaccessible due to strict privacy and anonymization protocols.

Clinical specificity

Clinical specificity evaluation was conducted separately for each assay using leftover patient samples (Table 1). HIV 1/2 Combo assays evaluation was performed using 1500 unknown patient samples, while the evaluation for the Syphilis assays was performed using 250 diagnostic leftover patient samples. All leftover patient samples used for clinical specificity evaluation were selected from anonymised, previously tested negative samples, collected during daily routine from apparently healthy subjects from Romania, with no clinical indication of HIV or any other infectious disease symptoms. All reactive samples were tested in duplicate on the analyzer that provided the positive test result. Reactive results were further investigated by additional confirmatory testing methods (PCR, RPR/VDRL/TPHA), together with available clinical information, where applicable, to determine whether an active infection was present or to classify the result as false 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 performance. 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).

Regarding sample size rationale, formal a priori power calculations were not performed because this study was designed as a non-interventional, post-market verification evaluation leveraging a prospective convenience cohort of residual clinical specimens. Instead, the sample sizes (1,500 negative patient samples for HIV specificity, 250 confirmed positive and 250 negative samples for syphilis, and 10 commercially sourced seroconversion panels of 132 individual specimens) were determined based on operational feasibility and the objective to meet or exceed international recommendations (CLSI and ISO 15189:2022) for laboratory immunoassay evaluations [15]. This study compared the three systems rather than formally establishing their primary baseline parameters. Given the global scarcity of commercial seroconversion panels, sample sizes were optimized for binomial precision; 1,500 HIV-negative samples ensure a 100% specificity estimate maintains a lower 95% confidence interval (CI) boundary of 99.75%, while 250 negative syphilis samples guarantee a lower 95% CI of 98.54%. This provides the high statistical precision needed to confidently isolate cross-platform performance differences.

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. A formal statistical correction for multiple comparisons was not applied to the primary diagnostic metrics, as this study focused primarily on estimating diagnostic metrics via standalone point estimates and exact confidence intervals, rather than testing multiple simultaneous null hypotheses or relying on a large network of exploratory p-values. Pairwise cross-platform metrics are reported directly as descriptive and absolute clinical performance differences to ensure unadjusted data transparency for laboratory selection. To formally evaluate specific cross-platform diagnostic gaps, pairwise differences in clinical sensitivity between platforms were evaluated using the exact McNemar's test for paired nominal data, with a significance threshold established at $\alpha = 0.05$. Because the analytical sensitivity panel dilutions were evaluated using 5 replicates per concentration level, a formal Probit regression analysis to calculate a statistical 95% Limit of Detection (LoD) curve was not mathematically feasible. Consequently, these endpoint sensitivity values are strictly presented as operational, panel-specific detection thresholds rather than formalized statistical LoD metrics.

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 deviation (SD). SD to cutoff ratio was calculated as the difference between the assay cutoff and the mean results signal, divided by the SD of the corresponding results distribution. A larger SD to cutoff value indicates greater separation from the cutoff and a lower likelihood of results misclassification due to analytical variation. This metric was used to assess how well the negative results were separated from the cutoff, thereby providing an estimate of assay robustness near cutoff and allowing comparison between assays independently of their reported signal units.

Additionally, the assessment of the reactive results distribution above assay cutoff assessment was included in order to determine the separation of the positive results relative to the cutoff, as low-positive results (gray-zone, borderline results) often fall within the analytical uncertainty range (more vulnerable to, and often caused by analytical interference). Thus, low positive results require a more cautious interpretive resolution than clearly reactive results located well above cutoff. The distribution of reactive results above the assay cutoff was plotted by grouping reactive sample results into value intervals relative to the cutoff (1.0-1.2, 1.2-1.4, 1.4-1.6, 1.6-1.8, 1.8-2.0, 2.0-3.0, 3.0-4.0, 4.0-5.0, and ≥5.0).

The proportion of reactive results within each interval was calculated using the total number of reactive results for that assay as the denominator.

Results

Precision evaluation results

The analytical precision of the three evaluated CLIA systems was determined by assessing both within-run repeatability and total within-laboratory precision across the evaluated assays (HIV 1/2 and Syphilis). Precision was quantified using the coefficient of variation (CV%) across replicates of multi-level control materials (Table 2A). All systems demonstrated acceptable overall precision profiles for routine diagnostic use. The obtained intermediate precision (CV%) was compared to the manufacturer's claimed performance (Table 2B).

For the HIV 1/2 Combo assays, Alinity i demonstrated excellent precision across all control levels (Table 2A), with measured precision (CV%) values below manufacturer claimed specifications (Table 2B). Maglumi X3 showed acceptable within-laboratory precision, with measured CV% values generally comparable to manufacturer claims, although higher than claimed variability was observed for several control levels. For CL-1200i, the measured CV% for the negative control (9.37%) exceeded the manufacturer claim (6.46%), it remained only marginally higher than the Alinity i (8.84%) and substantially lower than Maglumi X3 (17.32%), remaining within acceptable limits for qualitative infectious disease immunoassays. CL-1200i exhibited higher analytical precision than Maglumi X3 for both negative and positive antibody control materials (Table 2).

For the syphilis assays, all evaluated systems demonstrated acceptable within-laboratory precision under routine laboratory conditions (Table 2A). Measured precision (CV%) values were below manufacturer claimed limits for both control levels on Alinity i and Maglumi X3 (Table 2B). Measured CV% of the CL-1200i negative control (5.44%), although exceeding the manufacturer claim (3.29%), remained lower than the corresponding CV% observed for both Alinity i (11.07%) and Maglumi X3 (7.87%). Relatedly, the CV% of the CL-1200i for the positive control (2.30%) was only marginally higher than claimed (2.26%) and lower than observed variation for both Alinity i (2.83%) and Maglumi X3 (3.86%), While direct comparability is inherently limited by the use of distinct, manufacturer-specific control materials and varying concentrations, evaluating these overall trends indicates that the CL-1200i generally tended to exhibit the lowest overall imprecision across both control levels, whereas the Alinity i and Maglumi X3 demonstrated slightly higher variability, particularly near the negative baseline.

Table 2A: Precision evaluation results for HIV 1/2 Combo and Syphilis assays.

Assay	Material	Analyzer	N	Mean	Unit	Repeat. SD	Repeat. CV%	WithinLab SD	WithinLab CV%
HIV 1/2 Combo	Negative Control (-)	Alinity i	25	0.08	S/CO	0.005	6.00	0.007	8.84
		Maglumi X3	25	0.24	AU/mL	0.033	13.49	0.042	17.32
		CL1200i	25	0.1	COI	0.006	6.22	0.009	9.37
	Positive Control (1)	Alinity i	25	5.25	S/CO	0.068	1.30	0.068	1.30
		Maglumi X3	25	10.03	AU/mL	0.447	4.46	0.652	6.50
		CL1200i	25	4.86	COI	0.111	2.28	0.131	2.70
	Positive Control (2)	Alinity i	25	3.52	S/CO	0.067	1.90	0.078	2.21
		Maglumi X3	25	20.06	AU/mL	1.075	5.36	1.075	5.36
		CL1200i	25	8.11	COI	0.182	2.24	0.33	4.07
	Positive Control (3)	Alinity i	25	3.25	S/CO	0.044	1.37	0.054	1.66
		Maglumi X3	25	5.18	AU/mL	0.376	7.27	0.376	7.27
Syphilis	Negative Control (-)	Alinity i	25	0.04	S/CO	0.002	5.15	0.004	11.07
		Maglumi X3	25	0.31	AU/mL	0.023	7.52	0.024	7.87
		CL1200i	25	0.43	COI	0.010	2.46	0.023	5.44
	Positive Control (+)	Alinity i	25	2.55	S/CO	0.045	1.76	0.072	2.83
		Maglumi X3	25	10.28	AU/mL	0.350	3.40	0.40	3.86
		CL1200i	25	4.82	COI	0.109	2.27	0.111	2.30

Assay	Material	Analyzer	95% Lower SD	95% Lower CV%	95% Upper SD	95% Upper CV%
HIV 1/2 Combo	Negative Control (-)	Alinity i	0.005	6.12	0.013	15.86
		Maglumi X3	0.03	12.54	0.068	28.02
		CL1200i	0.006	6.45	0.016	17.09
	Positive Control (1)	Alinity i	0.053	1.02	0.095	1.81
		Maglumi X3	0.453	4.51	1.163	11.6
		CL1200i	0.098	2.01	0.200	4.11
	Positive Control (2)	Alinity i	0.058	1.66	0.117	3.33
		Maglumi X3	0.839	4.18	1.498	7.47
		CL1200i	0.218	2.68	0.681	8.39
	Positive Control (3)	Alinity i	0.040	1.23	0.084	2.59
		Maglumi X3	0.293	5.67	0.524	10.13
Syphilis	Negative Control (-)	Alinity i	0.003	7.09	0.009	24.83
		Maglumi X3	0.019	6.09	0.034	11.10
		CL1200i	0.015	3.47	0.053	12.36
	Positive Control (+)	Alinity i	0.049	1.92	0.138	5.39
		Maglumi X3	0.300	2.92	0.587	5.71
		CL1200i	0.086	1.79	0.155	3.21

Table 2B: Intermediate precision (Within-Lab CV%) compared to claimed values.

Assay	Control sample	N	Alinity i		Maglumi X3		CL1200i	
			Claimed	Measured	Claimed	Measured	Claimed	Measured
HIV 1/2 Combo	Negative Control	25	11.60%	8.84%	N/A	17.32%	6.46%	9.37%
	Positive Control 1	25	2.40%	1.30%	6.52%	4.46%	3.40%	2.70%
	Positive Control 2	25	5.00%	2.21%	4.65%	5.36%	3.66%	4.07%
	Positive Control 3	25	2.80%	1.66%	6.47%	N/A	N/A	N/A
Syphilis	Negative Control	25	12.10%	11.07%	N/A	7.87%	3.29%	5.44%
	Positive Control	25	4.40%	2.83%	4.29%	3.86%	2.26%	2.30%

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

Analytical sensitivity results for HIV 1/2 Combo

Analytical sensitivity results for the HIV 1/2 Combo assays for the 1st WHO HIV-1 p24 Ag panel (NIBSC 16/210) are summarized in Table 3. Alinity i HIV Combo Ab/Ag provided reactive results at every dilution tested for all subtypes/CRFs, including group O. Maglumi X3 remained positive down to 0.790 IU/mL for subtype B (panel member 16/214, dilution 1/10). CL-1200i returned positive results for antigen concentrations around 2.313 IU/mL, and reactive for group O at 2.05 IU/mL. For panel member 16/218 (subtype C) at dilution 1/15 corresponding to 0.767 IU/mL (not shown in Table 3), Maglumi X3 returned borderline negative results, very close to the assay cutoff (mean of 0.93 AU/mL for all 5 replicates, no result above assay cutoff), with reactivity close to cutoff (SD to cutoff 1.53, only 0.07 AU/mL below the cutoff). For panel member 16/222 (subtype F1/CRF12_BF/BFrec) at dilution 1/6 corresponding to a unitage of 2.12 IU/mL (not shown in Table 3), CL-1200i returned borderline negative results, very close to the assay cutoff (mean of 0.94 COI for all 5 replicates, no result above assay cutoff), indicating reactivity close to cutoff (SD to cutoff 1.74, only 0.06 COI below the cutoff). Analytical sensitivity evaluation revealed clear differences among the evaluated systems. Alinity i consistently demonstrated the highest analytical sensitivity, remaining reactive at the lowest tested antigen concentrations across all tested HIV-1 subtype samples. Maglumi X3 followed closely, demonstrating intermediate analytical sensitivity, while CL-1200i showed comparatively lower detection capability at low antigen concentrations.

Table 3: HIV-1 p24 Antigen analytical sensitivity of the HIV 1/2 Combo assays.

Panel members	Subtype	Unitage (IU/mL)	Alinity i	Maglumi X3	CL1200i
16/212	A1	8.8	Positive up to 1/12 (0.733 IU/mL)	Positive up to 1/10 (0.880 IU/mL)	Positive up to 1/4 (2.20 IU/mL)
16/214	B	7.9	Positive up to 1/12 (0.658 IU/mL)	Positive up to 1/10 (0.790 IU/mL)	Positive up to 1/3 (2.55 IU/mL)
16/216	B	8.4	Positive up to 1/11 (0.764 IU/mL)	Positive up to 1/10 (0.840 IU/mL)	Positive up to 1/3 (2.10 IU/mL)
16/218	C	11.5	Positive up to 1/16 (0.719 IU/mL)	Positive up to 1/14 (0.821 IU/mL)	Positive up to 1/5 (2.30 IU/mL)
16/220	D	9.0	Positive up to 1/12 (0.750 IU/mL)	Positive up to 1/11 (0.818 IU/mL)	Positive up to 1/4 (2.25 IU/mL)
16/222	F1/CRF12_BF/BFrec	12.7	Positive up to 1/18 (0.706 IU/mL)	Positive up to 1/16 (0.794 IU/mL)	Positive up to 1/5 (2.47 IU/mL)
16/224	G	8.0	Positive up to 1/11 (0.727 IU/mL)	Positive up to 1/10 (0.800 IU/mL)	Positive up to 1/3.5 (2.25 IU/mL)
16/226	CRF20_BG	9.9	Positive up to 1/14 (0.707 IU/mL)	Positive up to 1/12 (0.825 IU/mL)	Positive up to 1/4.5 (2.15 IU/mL)
16/228	CRF01_AE	10.3	Positive up to 1/14 (0.736 IU/mL)	Positive up to 1/13 (0.792 IU/mL)	Positive up to 1/4 (2.48 IU/mL)
16/230	CRF02_AG	4.0	Positive up to 1/5.5 (0.727 IU/mL)	Positive up to 1/5 (0.800 IU/mL)	Positive up to 1/1.5 (2.60 IU/mL)
16/232	H	6.0	Positive up to 1/8 (0.750 IU/mL)	Positive up to 1/7 (0.857 IU/mL)	Positive up to 1/2.5 (2.36 IU/mL)
16/234	group O	11.3	Positive up to 1/15 (0.753 IU/mL)	Positive up to 1/14 (0.807 IU/mL)	Positive up to 1/5.5 (2.05 IU/mL)
Mean unitage value (IU/mL) of antigen concentration for the last reactive dilutions across all panel members			**0.728 IU/mL**	**0.819 IU/mL**	**2.313 IU/mL**

Values in parentheses indicate the operational concentration of the final reactive dilution, calculated directly from the external WHO/NIBSC 16/210 panel member unitage and the applied dilution factor. Because these evaluated screeners are qualitative assays, these figures serve strictly as within-study relative endpoint reactivity comparisons under identical experimental conditions; they do not imply direct equivalence between the manufacturers' distinct master calibration frameworks (AFSSAPS mass-concentration for Alinity i vs. NIBSC 90/636 for Maglumi X3 and CL-1200i) nor do they represent formal platform LoD claims.

Potential endogenous interference evaluation results

For all the three CLIA systems, no clinically significant interference was observed from common endogenous substances tested according to CLSI EP07 (data not shown). Overall, Maglumi X3 immunoassays demonstrated slightly higher signal variation compared to Alinity i and CL-1200i. Although significant variations (in some cases exceeding ±10% relative difference between control and test pools) were observed in a few runs, the variation did not alter the qualitative interpretation of results (positive/negative). Notably, for HIV 1/2 Ab-Ag, Maglumi X3 showed concentration differences of 0.5-10.7% for creatinine (15 mg/dL), 0.8-14.9% for glucose (1000 mg/dL), 0.5-17.6% for glycerol (18 mg/dL), 3.3-13.7% for lactate (90 mg/dL) and 2.7-10.7% for uric acid (25 mg/dL) across negative, low positive, and positive pools, while CL-1200i showed differences of 3.7-15.1% for lactate (90 mg/dL). For all other endogenous metabolites, signal differences remained within ±10%. Accordingly, interference effects exceeding ±10% were not considered clinically significant as the qualitative results interpretation agreement between the control and test pools was maintained.

Clinical sensitivity results for HIV 1/2 Combo

Clinical sensitivity results for each individual HIV 1/2 seroconversion panel tested (10 panels), and the number of days since the 1st HIV positive duplicate test result is detected, are presented in Table 4. Across the HIV performance panel (data not shown), all 15 samples were identified as reactive by Alinity i and Maglumi X3. CL-1200i returned reactive results for 14/15 samples, with the exception of panel member 08 (0800-0492 [28]) - subtype C (data not shown), which was identified as reactive only by Alinity i and Maglumi X3 and not by CL-1200i.

Table 4: Days until 1st HIV detection (Seroconversion Panels).

No. Crt.	Seroconversion Panel ID	Alinity i	Maglumi X3	CL1200i
1	06000272	11	11	18
2	06000252	0	0	7
3	06000260	7	14	14
4	06000271	0	7	10
5	06000243	21	27	29
6	06000248	35	37	44
7	06000262	2	13	15
8	06000265	21	28	28
9	06000245	14	14	17
10	06000258	7	7	9
Mean number of days until detection		**11.8**	**15.8**	**19.1**
Number of positive results (out of 132 total specimens tested)		**78 (59.1%)**	**62 (46.9%)**	**46 (34.8%)**

Values shown for each seroconversion panel represent the day of the first reactive bleed detected by each assay. The mean number of days was calculated as the arithmetic mean of the day of first reactivity across all the 10 seroconversion panels for each assay. Number of positive results refers to the total number of reactive individual serial bleed panel member specimens detected by each assay out of 132 total specimens tested across all the seroconversion panels.

The clinical sensitivity evaluation revealed marked differences in early infection detection between the HIV-1/2 Combo assays (for panel-specific detection data, see Supplementary Table 2). Across the HIV panels (Table 4), Alinity i demonstrated the shortest average seroconversion period (mean of 11.8 days; median 9.0 with a range of 0-35), allowing HIV detection 7.3 days earlier compared to CL-1200i (mean of 19.1 days; median 16.0 with a range of 7-44), and 4 days earlier compared to Maglumi X3 (mean of 15.8 days; median 13.5 with a range of 0-37). Alinity i presented an increased average seroconversion sensitivity by 25% compared to CL-1200i, and by 12% compared to Maglumi X3, as Alinity i identified the highest proportion of early reactive results across seroconversion panels (59.1% positives detected). Maglumi X3 showed intermediate detection in selected panels (46.9% positives detected), while CL-1200i demonstrated lower average seroconversion sensitivity (34.8% positives detected) indicating a higher number of missed early infections relative to the other two assays. Although the assays differed in their overall mean time to detection and proportion of reactive specimens identified, several individual panel comparisons illustrate aligned performance during early HIV infection (p24 antigen detection).

The same 1st day of detection performance was observed for Alinity i and Maglumi X3 for four seroconversion panels: 0600-0272 (HIV-1 subtype B donor; 6 serial bleeds collected over 30 days); 0600-0252 (HIV-1 donor; 4 serial bleeds ranging from Fiebig Stage II to IV collected over 14 days); 0600-0258 (HIV-1 donor; 4 serial bleeds including Fiebig Stage I-II/III collected over 16 days); 0600-0245 (HIV-1 donor; 7 serial bleeds including Fiebig Stage I, II and II/III collected over 21 days). Notably, both Alinity i and Maglumi X3 detected early HIV infection: panel 0600-0252, starting with the 1st bleed (Day 0); panel 0600-0258, starting with 2nd bleed (Day 7); panel 0600-0245, starting with the 5th bleed (Day 14); all being equivalent to Fiebig Stage I (acute, early HIV infection) [39]. For Maglumi X3 and CL-1200i, the same 1st day of detection performance for early infection was observed for seroconversion panel 0600-0260 (HIV-1 donor; 5 serial bleeds including Fiebig Stage I to III collected over 14 days), starting with the 5th bleed (Day 14) equivalent to Fiebig Stage II (acute, early HIV infection) [39]. At these early stages (Fiebig I-II), infection is characterized by detectable plasma viral load HIV RNA PCR positive and p24 antigen positive (only by highly

sensitive 4th generation assays [40]), with negative HIV-1 antibody tests (as the immune system has not yet developed detectable antibodies) [41].

Clinical sensitivity results for syphilis

Clinical sensitivity results for the syphilis assays revealed comparable performance for Alinity i and Maglumi X3 (Table 5). Alinity i correctly identified 249 of 250 positive samples, corresponding to a clinical sensitivity of 99.60% (95% CI: 97.79-99.99%). Maglumi X3 correctly identified 248 of the 250 positive samples, corresponding to a clinical sensitivity of 99.20% (95% CI: 97.14-99.90%). In contrast, CL-1200i identified only 240 of 250 positive samples, obtaining a clinical sensitivity of 96.00% (95% CI: 92.77-98.07%), showing an increased rate of false negative results (10/250, 4%). Pairwise comparisons using the exact McNemar's test showed no statistically significant difference in sensitivity between Alinity i and Maglumi X3 (p = 1.000). However, both platforms demonstrated a statistically significant sensitivity advantage over the CL-1200i system (p = 0.012 for Alinity i vs. CL-1200i; p = 0.039 for Maglumi X3 vs. CL-1200i).

Table 5: Clinical sensitivity evaluation for syphilis assays.

Analyzer	Cutoff	Unit	N	Negative	Positive	Mean	Sensitivity	Sensitivity 95%CI
Alinity i	1	S/CO	250	1	249	13.93	99.60%	(97.79% / 99.99%)
Maglumi X3	1	AU/mL	250	2	248	85.85	99.20%	(97.14% / 99.90%)
CL1200i	1	COI	250	10	240	24.70	96.00%	(92.77% / 98.07%)

Although the majority of reactive samples on all systems were distributed well above the assay cutoff, the results distribution of results differed between assays (Supplementary Figure 1). The proportion of reactive results at ≥5.0 was 205/249 (82.3%) for Alinity i, 242/248 (97.6%) for Maglumi X3, and 220/240 (91.7%) for CL-1200i. Notably, Alinity i showed a higher proportion of low positive reactive results near the cutoff, with 15/249 (6%) reactive sample results falling between the value range of 1.2 and 2.2, compared with 3/248 (1.2%) for Maglumi X3 and 5/240 (2.1%) falling between the 1.0 and 2.2 value range for CL-1200i (Supplementary Figure 1). Maglumi X3 showed the greatest separation of reactive results from cutoff, with the highest proportion of strongly reactive results at ≥5.0 (242/248, 97.6%) and the lowest proportion of low-positive reactive results near cutoff in the 1.2-2.2 range (3/248, 1.2%). Alinity i showed the opposite pattern, with the lowest proportion of results above ≥5.0 (82.3%) and the highest proportion of reactive results clustered near cutoff (6%), while CL-1200i showed an intermediate pattern (91.7% results ≥5; 2.1% results between 1.0-2.2). Despite showing a greater proportion of reactive results clustered close to the cutoff, Alinity i demonstrated the highest clinical sensitivity, achieving reliable detection of the positive samples.

Clinical specificity evaluation results

Clinical specificity results are summarized in Table 6. For the HIV 1/2 Combo assays, from a total of 1,500 leftover patient samples tested, 12 samples were found reactive (returning duplicate positive results). All reactive samples were confirmed negative by confirmatory HIV-1/HIV-2 RNA PCR testing and were considered false positive results. Alinity i demonstrated a clinical specificity of 99.87% (95% CI: 99.52-99.98%), with 2 false reactive results (0.13% false positive rate), followed closely by Maglumi X3 and CL-1200i, both showed a specificity of 99.67% (95% CI: 99.22-99.89%), corresponding to 5 false reactive results for each system (0.33% false positive rate). The majority of negative samples across all HIV assays clustered well below the respective assay cutoffs (SD to cutoff 13.55 for Alinity i, 5.70 for Maglumi X3, 6.84 for CL-1200i) (Supplementary Figure 2A).

For syphilis, from a total of 250 leftover patient samples tested, 9 samples were found reactive on at least one of the analyzers. From these, two samples were found positive on two analyzers (one sample returned duplicate positive results on both Alinity i and CL-1200i, and another sample returned duplicate positive results on both Maglumi X3 and CL-1200i). All the reactive samples were confirmed negative by additional confirmatory testing (RPR/VDRL/TPHA) and were classified as false positive results. Both Alinity i and Maglumi X3 showed a specificity of 99.20% (95% CI: 97.14-99.90%), corresponding to 2 false reactive results (0.80% false positive rate). CL-1200i demonstrated a specificity of 97.20% (95% CI: 94.32-98.87%), corresponding to 7 false reactive results out of the 250 negative tested samples (2.80% false positive rate). The majority of negative samples across all assays clustered well below the respective assay cutoffs (SD to cutoff 4.15 for Alinity i, 2.35 for Maglumi X3, 1.47 for CL-1200i) (Supplementary Figure 2B).

Table 6: Clinical specificity for HIV 1/2 and Syphilis.

Assay	Analyzer	Cutoff	Unit	N	Negative	Positive	Mean	Specificity	Specificity 95%CI
HIV 1/2 Combo	Alinity i	1	S/CO	1500	1498	2	0.13	99.87%	(99.52% / 99.98%)
	Maglumi X3	1	AU/mL	1500	1495	5	0.21	99.67%	(99.22% / 99.89%)
	CL1200i	1	COI	1500	1495	5	0.12	99.67%	(99.22% / 99.89%)
Syphilis	Alinity i	1	S/CO	250	248	2	0.08	99.20%	(97.14% / 99.90%)
	Maglumi X3	1	AU/mL	250	248	2	0.33	99.20%	(97.14% / 99.90%)
	CL1200i	1	COI	250	243	7	0.48	97.20%	(94.32% / 98.87%)

Discussion

The rapid advancement and widespread implementation of CLIA platforms represents a critical milestone in the management of infectious diseases. However, the success of global public health strategies relies heavily on the real-world performance of these diagnostic tools. The need for accurate serological screening cannot be overstated, particularly given that syphilis continues to re-emerge globally and has been consistently associated with an increased risk of HIV acquisition [42]. Achieving the WHO goals to eliminate HIV, viral hepatitis, and sexually transmitted infections as public health threats by 2030 requires highly sensitive diagnostic frameworks. For instance, maternal syphilis, if untreated or treated too late, results in congenital infection in 50% to 80% of cases alongside severe adverse birth outcomes [9]. Similarly, early detection of HIV infection remains critical for blood safety and global elimination efforts. To address these diagnostic challenges, our study provides a comprehensive performance evaluation of the Alinity i, Maglumi X3, and CL-1200i systems under routine clinical laboratory conditions.

It is important to note that all three CLIA systems are deployed for high-throughput screening, they do not share identical cleared clinical applications. The Alinity i syphilis assay is explicitly indicated as a primary screening test to prevent infectious transmission via blood and tissues, whereas the manufacturers of the CL-1200i and Maglumi X3 systems explicitly exclude their use for evaluating blood donation or transplantation suitability [16,22,23]. These structural parameters offer an essential baseline context when analyzing the variations in performance across the systems.

Our findings revealed that all tested CLIA platforms exhibit acceptable overall precision profiles for routine diagnostic use. Instances in which manufacturer claimed precision specifications were not fully met likely reflect differences between controlled manufacturer validation environments and routine clinical laboratory conditions rather than clinically meaningful analytical limitations. For example, relatively higher variation observed at the negative control level remained well below assay cutoff thresholds and did not impact clinical interpretation. Furthermore, endogenous interference testing confirmed the robustness of the tested CLIA systems against common biochemical interferents that could be found in patients with metabolic disturbances or renal dysfunction.

Analytical sensitivity in HIV screening is primarily driven by the ability to detect low-level p24 antigen during the early phase of infection, prior to the development of a fully established antibody response. Evaluating assay performance using progressive dilutions of diverse, recombinant HIV panels is critical because it rigorously simulates the low biomarker concentrations characteristic of acute or suppressed infections across a globally representative array of viral subtypes [11,43]. Because these pharmacological interventions can profoundly blunt p24 antigenemia and delay antibody maturation, individuals experiencing breakthrough infections often present with atypical, highly suppressed serological profiles. Earlier detection during seroconversion is particularly important for blood safety screening, as p24 antigen may be the only detectable marker before antibody maturation.

Our evaluation revealed clear performance differences, with the Alinity i platform allowing on average HIV detection at least 7.3 days earlier compared to CL-1200i, and at least 4 days earlier compared to Maglumi X3. This cross-platform performance hierarchy was fully supported by median values (Alinity i: 9.0 days; Maglumi X3: 13.5 days; CL-1200i: 16.0 days). Furthermore, a leave-one-out sensitivity analysis (data not shown) confirmed that no single panel disproportionately drove these observed variations, with baseline mean differences shifting by less than 1.0 day across all iterations. Such differences are likely attributable to variations in assay design and the specific biological reagents employed (e.g. recombinant antigens and monoclonal antibodies) alongside differences in signal amplification chemistry and detection kinetics among the systems. When interpreting these variations, the underlying metrological non-equivalence of the assays' baseline calibration standards must be considered. The Alinity i system relies on traceability to AFSSAPS mass-concentration definitions (<50 pg/mL of p24), whereas the Maglumi X3 and CL-1200i platforms are calibrated against NIBSC 90/636 international activity units (IU/mL). While utilizing a standardized external reference panel (WHO/NIBSC 16/210) provides an operational look at relative endpoint reactivity under identical conditions, these distinct manufacturing calibration frameworks prevent direct numerical translation and reinforce why these metrics reflect panel-specific performance rather than interchangeable absolute sensitivities.

Ultimately, these variations highlight key performance

characteristics with potential clinical implications for early infection identification. Maximized analytical sensitivity across diverse viral subtypes is essential not only for monitoring specific clinical cohorts but for routine diagnostic testing across all settings. The ability to reliably detect low-level biomarkers during the earliest stages of infection is vital for guiding prompt treatment initiation, optimizing patient outcomes, and implementing effective public health strategies to prevent ongoing HIV transmission. This clinical significance is further clarified by valuating performance against the panels' manufacturer-verified NAT data. Tracking the residual diagnostic window (i.e. the days between initial RNA detection and immunoassay reactivity) reveals clear cross-platform gaps. High-sensitivity systems often closed the serological window immediately (0 days) or within 4 days of NAT positivity, whereas less sensitive architectures left extended diagnostic gaps of up to 7 to 11 days.

The clinical relevance of sensitivity differences must also be interpreted in the context of the global re-emergence of syphilis. In such settings, even modest reductions in assay sensitivity may translate into a meaningful number of missed infectious cases, with implications for transmission control, prenatal screening and congenital syphilis prevention. From a clinical and public health perspective, failure to detect 4% of confirmed positive samples represents a meaningful diagnostic gap for the CL-1200i platform. This performance tiering was statistically verified by the exact McNemar's test; while Alinity i and Maglumi X3 share statistical equivalence, both platforms achieved a statistically superior diagnostic threshold over the CL-1200i architecture.

The observed differences underscore the importance of assay selection in clinical contexts where early detection is critical. Indeed, as mentioned previously, this analytical divergence directly aligns with the CLIA systems' underlying intended use scopes [16,22,23]. Additionally, because syphilis CLIA immunoassays can be used in reverse diagnostic algorithms, discordant screening results necessitate downstream non-treponemal and treponemal confirmatory testing to accurately differentiate active infections. Specifically, the intended use statements for Maglumi X3 warn about these limitations [22]. Clinical specificity was highly preserved across all platforms, though minor variations were observed. In high volume screening environments, even small differences in false positive rates may have operational implications, including increased confirmatory testing, and additional laboratory workload. Together, our results show that post-market evaluations can be very useful in revealing real-world performance differences that may not be fully captured during manufacturer in-house validation, particularly under the regulatory framework established by the EU IVDR [44]. Our study provides a robust and comprehensive evaluation of infectious disease immunoassays under authentic clinical laboratory conditions. By utilizing highly characterized WHO reference materials, standardized seroconversion panels, and a large cohort of clinical diagnostic samples, our assessment yields highly reliable and actionable insights into the real-world performance of the CLIA systems. These assays offer same-day results aiding in diagnosis more swiftly than western blotting (HIV) and RPR/VDRL/TPHA (syphilis), thereby reducing the percentage of loss to follow-up [45,46]. For HIV, WHO testing strategies recommend national algorithms that use immunoassays instead of nucleic acid testing techniques and western blotting for the diagnosis of subjects over 18 months of age [45]. Similarly, for syphilis, the high-throughput capabilities of automated CLIAs have driven a global shift toward the "reverse" screening algorithm. By utilizing a highly sensitive treponemal immunoassay as the initial screening step rather than a traditional non-treponemal test like RPR, the reverse algorithm significantly improves the detection of early primary and late latent infections while reducing overall laboratory costs and turnaround times [46,47]. Ultimately, improving throughput capacity and time-to-result across both targets facilitates rapid linkage to prevention and treatment services, directly supporting the WHO goal of ending sexually transmitted infections as public health threats by 2030[3].

Nevertheless, certain limitations must be acknowledged. The generalizability of our findings is bounded by the study's single-center design utilizing a regional Romanian patient cohort, alongside the omission of specific manufacturing reagent lot numbers and instrument software versions. While these omissions restrict absolute longitudinal replication across diverse global demographics, the single-site framework successfully isolated the platforms from inter-laboratory environmental noise. Furthermore, genotype diversity was inherently limited to the available commercial panels and reference materials. Similarly, the lack of clinical staging, historical treatment documentation, and individual reference titer values for the syphilis-positive cohort prevents a stage-specific sensitivity analysis or a definitive clinical root-cause determination for the observed assay failures. Additionally, a limitation of the endogenous interference assessment was the utilization of pooled patient sera rather than individual clinical samples. While this pooling approach was operationally mandatory to secure the high sample volumes (~20 mL per interferent) required for parallel testing across three automated platforms, and directly complied with the commercial kit's standardized protocol for comparing baseline and spiked matrices, it inherently minimizes individual patient matrix variability near the assay cutoff thresholds. Lastly, parameters such as long-term reagent stability and lot to lot variability were not explicitly evaluated. Consequently, the reported performance characteristics strictly reflect the specific testing conditions present during the study period and may exhibit temporal or geographic variations.

Despite these constraints, the foundational evidence presented here can provide valuable guidance for CLIA platform selection and optimizing blood safety protocols. While all evaluated systems demonstrated acceptable precision and robustness for

routine laboratory use, their diagnostic sensitivity capabilities varied considerably. Specifically, the Alinity i system provided the earliest detection of HIV seroconversion, whereas the CL-1200i platform exhibited a notable diagnostic gap in clinical syphilis sensitivity. Ultimately, aligning system capabilities with strict clinical intended uses is essential to support global public health initiatives and infectious disease elimination targets. Future research that incorporates multicenter evaluations and longitudinal stability assessments have the potential to provide more information and extend the findings of the comparative assessment presented herein. Furthermore, studies aimed at targeted prospective evaluations within PrEP-using and early-ART cohorts may provide important information on how these analytical variations impact patient outcomes. Ultimately, continuous real-world validation frameworks are essential for maintaining diagnostic excellence and complying with stringent regulatory requirements.

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.

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Data availability statement

The analyzed data that support the findings of this study are presented in the article and included in the supplementary materials, detailed datasets and further information inquiries 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 D.C., S.D., 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.

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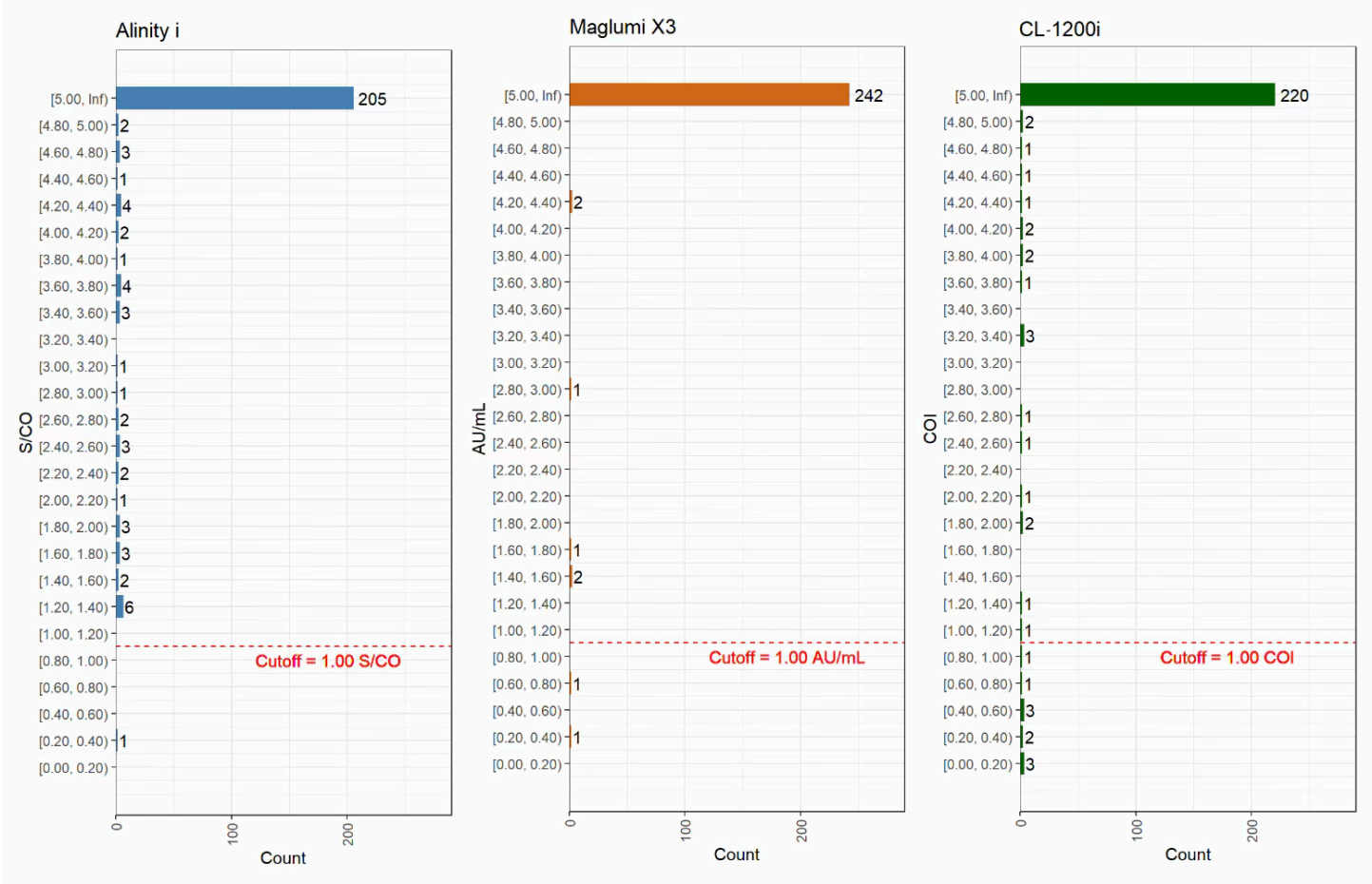
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Supplementary Table 1: Characteristics and technical specifications of each system.

	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)	Chemiluminescent microparticle immunoassay (CMIA)	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

CLIA (chemiluminescent immunoassay), ABEI [N-(4-Aminobutyl)-N-ethylisoluminol], NaOH (sodium hydroxide), H2O2 (hydrogen peroxide), AMPPD [3-(2`-spiroadamantane)-4-methoxy-4-(3``-phosphoryloxy)phenyl-1, 2-dioxietane disodium salt], ALP (Alkaline phosphatase).

Supplementary Figure 1: Distribution of the results obtained for the clinical sensitivity evaluation of the syphilis antibody assays on the three automated CLIA platforms (Alinity i, Maglumi X3, and CL-1200i).



The figure summarizes results for 250 residual serum samples confirmed positive for syphilis by reference serology (RPR/VDRL/TPHA). In each panel, horizontal bars indicate the number of samples within each signal values interval, and the dashed red line indicates the reactive assay cutoff. Results are reported in assay specific units (S/CO for Alinity i, AU/mL for Maglumi X3, and COI for CL-1200i); therefore, the distributions should be interpreted mainly by their separation from the cutoff within each platform, rather than by direct comparison of absolute numeric values across analyzers. Most positive samples were strongly reactive, with results ≥5.0 observed in 205/249 reactive samples (82.3%) on Alinity i, 242/248 (97.6%) on Maglumi X3, and 220/240 (91.7%) on CL-1200i. Low-positive results near the cutoff were more frequent on Alinity i, with 15/249 reactive samples (6.0%) between 1.2 and 2.2, compared with 3/248 (1.2%) on Maglumi X3 and 5/240 (2.1%) on CL-1200i. Samples falling below the cutoff represent false-negative results detected on each assay (1/250 on Alinity i, 2/250 on Maglumi X3, and 10/250 on CL-1200i), respectively, corresponding to clinical sensitivities of 99.60% (Alinity i), 99.20% (Maglumi X3) and 96.00% (CL-1200i). Specifically, the 10 false-negative specimens on the CL-1200i platform were distributed across the following non-reactive value ranges: 3 specimens within [0.00, 0.20) COI, 3 specimens within [0.20, 0.40) COI, 3 specimens within [0.40, 0.60) COI, and 1 specimen within [0.80, 1.00) COI. The profound suppression of signal (≤0.60 COI) in 90% of these missed cases indicates a structural absence of antibody recognition rather than borderline imprecision, likely corresponding to early primary infections or historical, successfully treated latent cases with low residual antibody titers. Overall, the figure shows that Maglumi X3 produced the tightest clustering of strongly reactive results, whereas Alinity i, despite a larger fraction of near cutoff positives, achieved the highest overall sensitivity.

Supplementary Figure 2: Distribution of the results obtained for the clinical specificity evaluation of the HIV 1/2 Combo assays (A) and syphilis assays (B) on the three automated CLIA platforms (left to right: Alinity i, Maglumi X3, and CL-1200i).



Horizontal bars show the number of results within each interval; the dashed red line marks the reactive assay cutoff. Panel (A) summarizes results for 1,500 HIV negative residual samples, corresponding to clinical specificities of 99.87% for Alinity i (2/1500 false positives), 99.67% for Maglumi X3 (5/1500), and 99.67% for CL-1200i (5/1500). The SD to cutoff (13.55 for Alinity i, 5.70 for Maglumi X3, and 6.84 for CL-1200i) indicating the greatest negative result separation on Alinity i. Panel (B) summarizes results for 250 syphilis negative residual samples. Specificity was 99.20% for Alinity i (2/250 false-positives), 99.20% for Maglumi X3 (2/250) and 97.20% for CL-1200i (7/250); the corresponding SD to cutoff values were 4.15, 2.35, and 1.47, respectively. Samples above cutoff represent false reactive results (resolved by confirmatory testing). This figure therefore illustrates not just overall specificity, but also how the negative results are separated from the reactive cutoff threshold.

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