

Analytical and Clinical Performance Evaluation of the Elecsys HIV combi PT Assay on the cobas e 602 Analyzer for the Diagnosis of Human Immunodeficiency Virus

Denise L. Uettwiller-Geiger, PhD,¹ Marvin Lessig, DO,² Jie An, PhD,³ Tara Barsch,¹ Susan Smith,⁴ Sharan Walker,⁴ Alexander Riedel, PhD,⁵ Yi Hao, MSc,⁵ and Amin A. Mohammad, PhD³

From the ¹Mather Hospital Northwell Health, Port Jefferson, NY; ²Nationwide Laboratory Services, Boca Raton, FL; ³Baylor Scott & White Health, Temple, TX; ⁴Roche Diagnostics, Indianapolis, IN; and ⁵Roche Diagnostics, Penzberg, Germany.

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ABSTRACT

Objectives: We evaluated the performance of the Elecsys HIV combi PT assay on the cobas e 602 analyzer for diagnosing human immunodeficiency virus (HIV; part of the US Food and Drug Administration [FDA] submission).

Methods: The HIV combi PT and reference (ARCHITECT HIV Ag/Ab Combo) assays were assessed at four independent clinical laboratories/one reference laboratory (United States; July 2014 to November 2015). Clinical performance was evaluated using four reagent lots. Analytical performance was evaluated per Clinical and Laboratory Standards Institute EP05-A3 guidelines. Serum/plasma samples from 18 clinical sites/vendors (United States and outside the United States) were tested.

Results: Sensitivity (95% confidence interval [CI]) in HIV-1 antibody-positive individuals (United States and outside the United States; $n = 1,460$) was 100.00% (99.75%-100.00%). Specificity was 99.94% (95% CI, 99.85%-99.98%) in low-risk individuals (United States; $n = 6,843$), 98.19% (95% CI, 96.93%-99.04%) in high-risk individuals (United States and outside the United States; $n = 758$), and 97.43% (95% CI, 95.32%-98.76%) in pregnant women (United States and outside the United States; $n = 440$). Analytical performance was acceptable.

Conclusions: We demonstrate the robustness of the FDA-approved Elecsys HIV combi PT assay on the cobas e 602 analyzer for HIV testing in the United States.

Over 36 million people have human immunodeficiency virus (HIV) worldwide, with approximately 2 million new cases annually.¹ In the United States, approximately 1.2 million people have HIV, of whom 13% remain undiagnosed.² The two main strains of HIV are HIV-1, categorized into groups M (the most common group, with nine subtypes), O, N, and P, and HIV-2.¹ In areas where diverse subtypes are prevalent, HIV diagnostic assays must be sensitive to the full range of groups and subtypes. Early viral identification facilitates timely initiation of antiretroviral therapy, which can increase life expectancy.³ Therefore, reliable HIV screening is important to reduce the human and financial burden associated with HIV transmission.⁴

HIV diagnosis usually requires detection of the HIV-1 p24 antigen or antibodies to HIV-1 and HIV-2, and confirmatory tests are performed according to Centers for Disease Control and Prevention (CDC) recommendations.^{5,6} The Elecsys HIV combi PT assay (fourth generation; Roche Diagnostics) is a qualitative, serologic sandwich assay intended for simultaneous in vitro determination of HIV-1 p24 antigen and antibodies to HIV-1 (including group O) and HIV-2 in human serum or plasma. Unlike previous Elecsys HIV combi assays, the Elecsys HIV combi PT assay includes a pre-treatment step, which enhances sensitivity and specificity for the HIV-1 p24 antigen and improves early detection of HIV seroconversion. The Elecsys HIV combi PT assay has been shown to reliably detect all known HIV subtypes using the Elecsys 2010 (Roche Diagnostics), cobas e 411 (Roche Diagnostics), or MODULAR E170

(Roche Diagnostics) analyzers.⁷ The assay has also been validated for screening and detection of HIV in Asia and is Conformité Européenne approved for use in Europe.^{7,8}

This study aimed to validate the performance of the Elecsys HIV combi PT assay on the cobas e 602 system (Roche Diagnostics) under routine laboratory conditions in the United States (part of the US Food and Drug Administration [FDA] submission).

The walkaway, freestanding cobas e 602 immunoassay analyzer (throughput 170 tests per hour) is compatible with 61 assays and can simultaneously test 25 different biomarkers in either random access or batch mode. The analyzer is equipped with onboard reagent refrigeration and dilution, as well as clot/serum indices (icterus, hemolysis, and lipemia) detection, and capable of over 16 hours of continuous testing per day. Autocalibration, onboard quality control monitoring, and data storage facilitate data tracking and maintaining study logs.

Materials and Methods

Study Design

Three geographically diverse laboratories in the United States (John T. Mather Memorial Hospital, Port Jefferson,

NY; Baylor Scott & White Health, Temple, TX; and Nationwide Laboratory Services, Boca Raton, FL) tested a total of 9,899 different samples with the Elecsys HIV combi PT assay on the cobas e 602 analyzer between July 2014 and November 2015. Details of the samples tested by each laboratory are shown in **Table 1**. Two additional laboratories in the United States (Quest Diagnostics, Valencia, CA, and ZeptoMetrix, Buffalo, NY) performed reference and confirmatory testing. Serum/plasma samples were collected at 18 clinical sites or purchased from vendors between July 2013 and October 2015. The clinical performance, analytical performance, and seroconversion detection sensitivity of the Elecsys HIV combi PT assay were evaluated. Inclusion criteria for the cohorts tested are provided in **Supplementary Table 1** (all supplemental materials can be found at *American Journal of Clinical Pathology* online). Analytical specificity and drug interference assessments were performed at Roche Diagnostics (Penzberg, Germany).

Samples were collected according to common/local ethical principles and were deidentified before testing. The study was conducted in accordance with applicable regulations, the principles of the Declaration of Helsinki, and the Convention of the Council of Europe. All sites received institutional review board approval from John T. Mather Memorial Hospital Institutional Review Board,

Table 1
Samples Tested at Each Laboratory

Cohort ^a	No. (%) of Samples Tested			Total
	John T. Mather Memorial Hospital	Baylor Scott & White Health	Nationwide Laboratory Services	
US cohorts				
Adults at low risk for HIV	2,159 (36)	1,622 (27)	2,269 (38)	6,050 (100)
Adults at high risk for HIV	151 (30)	176 (35)	172 (34)	499 (100)
Confirmed HIV-1–positive adults	414 (40)	401 (39)	210 (20)	1,025 (100)
Pregnant women at high risk for HIV	41 (53)	37 (47)	0	78 (100)
Confirmed HIV-positive pregnant women	11 (44)	14 (56)	0	25 (100)
Confirmed HIV-negative pregnant women	62 (31)	76 (38)	64 (32)	202 (100)
Children/adolescents at low risk for HIV	139 (24)	444 (75)	8 (1)	591 (100)
Children/adolescents at high risk for HIV	39 (29)	83 (62)	12 (9)	134 (100)
Confirmed HIV-1–positive children/adolescents	27 (56)	21 (44)	0	48 (100)
Other infectious viral diseases	35 (50)	35 (50)	0	70 (100)
Non-US cohorts or obtained from commercial vendors				
Confirmed HIV-1–positive adults	94 (47)	106 (53)	0	200 (100)
Pregnant women at high risk for HIV	20 (43)	27 (57)	0	47 (100)
Confirmed HIV-positive pregnant women	20 (57)	15 (43)	0	35 (100)
Confirmed HIV-1–positive children/adolescents	0	2 (100)	0	2 (100)
HIV-2 endemic area	242 (34)	241 (34)	223 (32)	706 (100)
HIV-1 group O	16 (38)	26 (62)	0	42 (100)
HIV-1 group M subtypes	37 (49)	38 (51)	0	75 (100)
Antigen specimens	25 (50)	25 (50)	0	50 (100)
HIV-1 seroconversion	10 (50)	10 (50)	0	20 (100)
Total	3,542 (36)	3,399 (34)	2,958 (30)	9,899 (100)

HIV, human immunodeficiency virus.

^aAdults and HIV-negative pregnant women were 21 years or older; other pregnant women cohorts were 18 years or older; children/adolescents were aged 2 or more to less than 22 years. Although the inclusion criteria in the study protocol specified age 22 years or older for adult and pregnant women cohorts, some individuals aged 18 to 21 years were included in these cohorts under a waiver; however, most adults and pregnant women were 22 years or older.

Baylor Scott & White Health Institutional Review Board, Western Institutional Review Board (for Nationwide Laboratory Services), and Copernicus Group (for Quest Diagnostics) to conduct the study.

Objectives

Primary objectives were to determine the clinical performance of the Elecsys HIV combi PT assay on the cobas e 602 analyzer relative to final diagnosis (determined by the recommended confirmation algorithm^{9,10} and independent of the Elecsys HIV combi PT assay result) and the positive percent agreement (PPA) and negative percent agreement (NPA) between the Elecsys HIV combi PT assay and reference assay (ARCHITECT HIV Ag/Ab Combo assay, fourth generation; Abbott Laboratories). Secondary objectives were analytical performance, seroconversion detection sensitivity (relative to the reference assay), and analytical specificity (in populations with other viral diseases and confirmed HIV-negative pregnant women) of the Elecsys HIV combi PT assay on the cobas e 602 analyzer.

Assays

The Elecsys HIV combi PT assay is a qualitative serologic, three-incubation step sandwich assay (total assay time 27 minutes; further details on the assay test principle are in the [Supplementary Appendix](#)). Results are determined automatically by the cobas e 602 analyzer by comparing the electrochemiluminescence signal from the sample with the cutoff value determined by calibration. Samples with a cutoff index (COI) less than 1.0 are nonreactive and were considered negative for HIV-1 p24 antigen and antibodies to HIV-1 and HIV-2; no further testing is required. Samples with a COI of 1.0 or more were considered reactive. These initially reactive samples were retested in duplicate with the Elecsys HIV combi PT assay; any samples with a COI of 1.0 or more in either retest were considered repeatedly reactive. These samples were confirmed according to the CDC-recommended confirmatory algorithm.^{9,10} See [Figure 1](#) for the HIV testing algorithm used in this study. All known HIV-positive samples were confirmed with a reactive HIV-1/2 Western blot and compared with the final diagnosis determined by the HIV testing algorithm.

Manufacturer instructions and results reporting procedures were followed for both the Elecsys HIV combi PT and ARCHITECT HIV Ag/Ab Combo assays.

Clinical Performance

The Elecsys HIV combi PT assay was evaluated on the cobas e 602 analyzer at three clinical laboratories using

four reagent lots. Samples (United States and outside the United States) included individuals at low and high risk of HIV (adults and children/adolescents), pregnant women, and individuals confirmed positive for specific HIV groups (including groups O and M, as well as HIV-2). Samples were also evaluated on the reference assay and additional assays/methods used in the testing algorithm ([Figure 1](#)).

Fresh samples (low-risk cohorts) were tested on the Elecsys HIV combi PT assay and reference assay within 72 hours of collection. Frozen samples (-20°C or lower) were randomized and distributed approximately equally among testing sites, which were blinded to sample/donor information. Samples that were reactive on the Elecsys HIV combi PT assay and had a final diagnosis of HIV negative underwent confirmatory analysis, including individual testing against HIV-1, HIV-2, and p24 antigen. Samples nonreactive for HIV-1, HIV-2, and p24 antigen individually but reactive on the Elecsys HIV combi PT assay were deemed false positives. It should be noted that a reactive result on the Elecsys HIV combi PT assay does not differentiate between reactivity of the HIV-1 p24 antigen or of antibodies to HIV-1 and HIV-2.

Analytical Performance

Repeatability (within-run precision), intermediate precision (within-laboratory precision), and reproducibility (between-laboratory precision) were evaluated according to Clinical and Laboratory Standards Institute (CLSI) EP05-A3 guidelines.¹¹ Experiments were performed at three sites using cobas e 602 analyzers with three reagent lots and one lot each of PreciControl HIV Gen II (levels 1-3) and PreciControl HIV; HIV-2+ GrpO (levels 4-5). Sample pools included PreciControl HIV levels 1 to 5, eight spiked human serum pools, and dummy samples (laboratory deidentified, leftover samples). Samples were analyzed in random order during two runs per day (two replicates of each sample per run) for 21 days.

Seroconversion Detection Sensitivity

Twenty seroconversion panels, obtained from SeraCare Life Sciences and ZeptoMetrix, comprising samples taken from patients at multiple time points before and after they tested positive for HIV, were tested with the Elecsys HIV combi PT assay and reference assay.

Analytical Sensitivity/Specificity

The Elecsys HIV combi PT assay was designed to have an analytical sensitivity of 2 IU/mL or less using the First International Standard HIV-1 p24 Antigen (National Institute for Biological Standards and Control

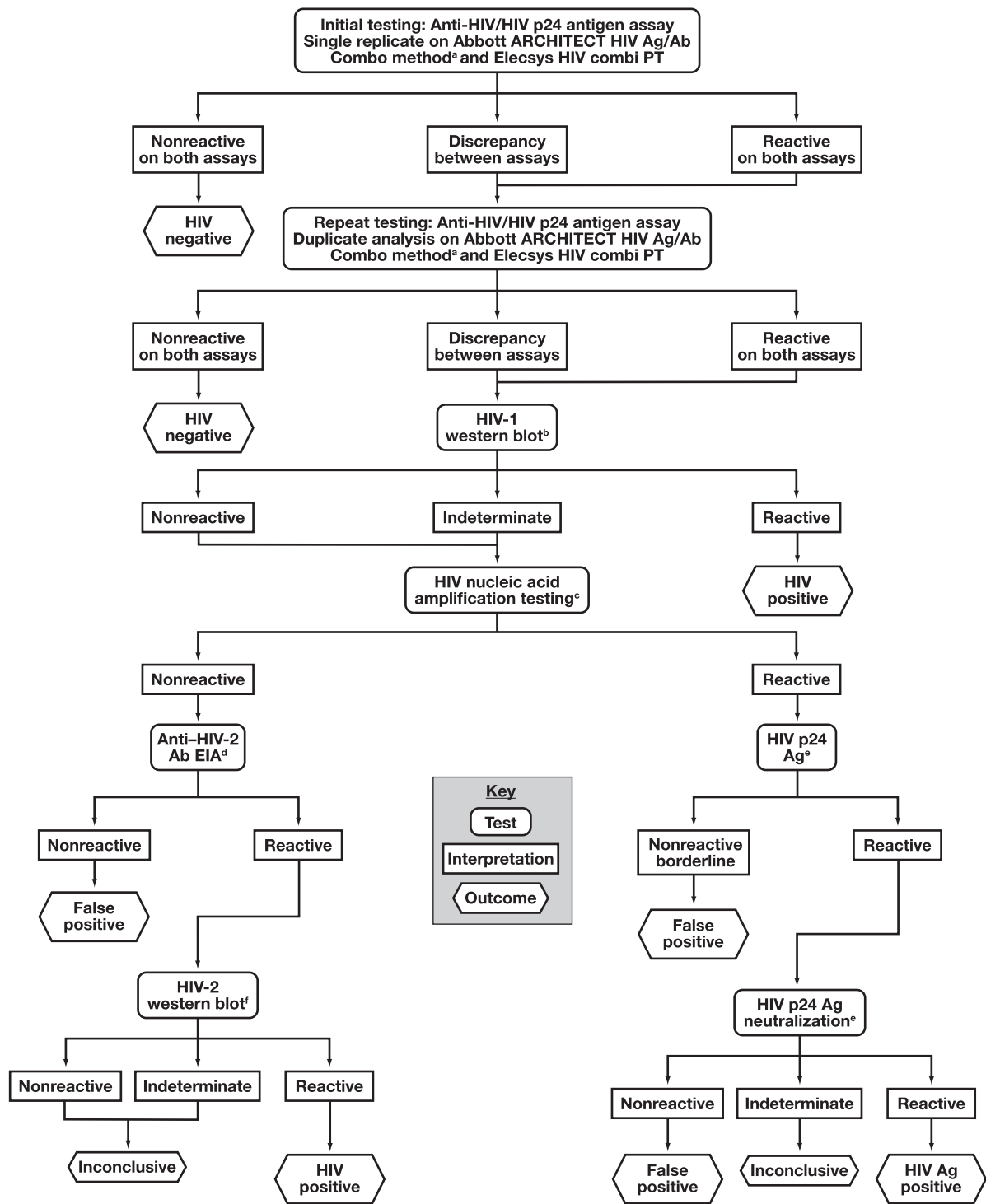


Figure 1 HIV testing algorithm. Algorithm for the method comparison of the Elecsys HIV combi PT assay with comparator HIV testing methods. Ab, antibody; Ag, antigen; EIA, enzyme immunoassay; HIV, human immunodeficiency virus. ^aUS Food and Drug Administration–approved HIV Ag/Ab reference assay. ^bBio-Rad Genetic System. ^cAbbott Real Time. ^dBio-Rad Genetic Systems. ^eZeptoMetrix. ^fImmunitics QualiCode.

code 90/636). In an internal study, the standard was diluted with HIV-negative serum. Using three lots of reagent, six dilution steps for each standard were prepared and measured in duplicate.

Analytical specificity of the Elecsys HIV combi PT assay was tested using samples from individuals with other viral diseases/medical conditions, which were selected on the basis that they are common HIV coinfections or

conditions that may potentially interfere with the assay (further details are in the [Supplementary Appendix](#)). Testing was conducted neat (unspiked; specificity testing) and with aliquots individually spiked with p24 antigens and antibodies to HIV-1 and HIV-2 (sensitivity testing). All experiments were performed on a cobas e 602 analyzer. Confirmatory testing was not performed.

Potential Interfering Analytes, Endogenous Substances, and Therapeutic Drugs

The Elecsys HIV combi PT assay was evaluated for potential cross-reactivity using samples from individuals known to be infected with hepatitis A (acute, recovered, or chronic), B, and C; rubella virus; cytomegalovirus; Epstein-Barr virus; or herpes simplex virus. Ten samples from each viral disease state were spiked to near-cutoff values with p24 antigen and antibodies to HIV-1 and HIV-2. All samples were randomized and tested both spiked and neat (unspiked).

Interference from endogenous substances (including biotin) and 18 therapeutic drugs (at concentrations 3-10 times the maximum clinically indicated dosage, per CLSI EP07-A2 guidelines¹²) was evaluated (see [Supplementary Table 2](#)). Each drug was spiked into a negative, anti-HIV antibody-positive (signal/cutoff ratio 2-4) and HIV antigen-positive (signal/cutoff ratio 2-4) sample (three replicates per drug per sample), respectively, and evaluated with the assay.

Statistical Analyses

Data from the Elecsys HIV combi PT assay were captured by Windows-based computer-aided evaluation software (WinCAEv; Roche Diagnostics). Data from reference and confirmatory assays were entered offline into WinCAEv. Clinical information was entered into an electronic database (Medrio version 9.0) at sample collection sites (prospectively collected) or at Roche (commercially acquired samples). Statistical analyses were performed using R software (R Foundation for Statistical Computing) and SAS (SAS Institute).

Imprecision was assessed for positive and negative pools by calculating coefficient of variation (CV) and SD. Acceptance criteria for positive pools were repeatability (CV \leq 6%), intermediate precision (CV \leq 10%), and reproducibility (CV \leq 25%). Acceptance criteria for negative pools were repeatability (COI $<$.6, SD $<$.06), intermediate precision (COI $<$.6, SD $<$.09), and reproducibility (COI $<$.9, SD $<$.15). Acceptance criteria for the entire imprecision study were 95% or more of all precision results within the acceptance criteria.

To assess clinical performance, PPA and NPA were calculated by comparing Elecsys HIV combi PT assay

results with the reference assay; sensitivity and specificity were calculated by comparing Elecsys HIV combi PT assay results with the final diagnosis. Data were presented as point estimates and 95% confidence intervals (CIs). Further details on the statistical analyses are in the [Supplementary Appendix](#), including [Supplementary Tables 3 and 4](#).

A list of all study cohorts, pooled data sets, and subgroups is shown in [Supplementary Table 5](#).

Results

Clinical Sensitivity

Reactivity in different study cohorts using the Elecsys HIV combi PT assay and reference assay is shown in [Table 2](#). Overall sensitivity of the Elecsys HIV combi PT assay in all confirmed HIV-1–positive individuals (United States and outside the United States; $n = 1,460$, including adults, children/adolescents, and HIV-positive pregnant women) was 100.00% (95% CI, 99.75%-100.00%). Among individuals at high risk for HIV ($n = 758$, including adults [United States], children/adolescents [United States], and pregnant women [United States and outside the United States]), 51 and 41 were repeatedly reactive using the Elecsys HIV combi PT assay and reference assay, respectively, and 38 were confirmed positive by Western blot. Overall sensitivity of the Elecsys HIV combi PT assay in the HIV-2 endemic area cohort was 99.64% (276/277; 95% CI, 98.01%-99.99%); sensitivity of the Elecsys HIV combi PT assay in confirmed HIV-2–positive individuals ($n = 211$) was 100.00% (95% CI, 98.27%-100.00%).

All samples from confirmed HIV-1–positive children/adolescents (United States and outside the United States; $n = 50$) were repeatedly reactive using both the Elecsys HIV combi PT assay and reference assay. Of 134 samples from children/adolescents at high risk for HIV (United States), one was repeatedly reactive using both the Elecsys HIV combi PT assay and reference assay, as well as confirmed positive by Western blot ([Table 2](#); [Supplementary Table 6](#)).

All 60 samples from HIV-positive pregnant women (United States and outside the United States) were repeatedly reactive using the Elecsys HIV combi PT assay and reference assay. Nine of the 10 reactive samples from high-risk pregnant women that were not confirmed as HIV positive originated from outside the United States. In high-risk pregnant women (United States), specificity was 98.53% (67/68; 95% CI, 92.08%-99.96%) and sensitivity was 100.00% (10/10; 95% CI, 69.15%-100.00%). In high-risk pregnant women (outside the United States), specificity was 68.75% (22/32; 95% CI,

Table 2
Reactivity in Different Study Cohorts

Cohort ^a	No. of Samples Tested	Eleclys HIV combi PT Assay, No.			Reference Assay, No.		
		NR	IR	RR	NR	IR	RR
Confirmed HIV-1–positive individuals							
US adults							
Asymptomatic adults	820	0	820	820	0	820	820
Symptomatic adults	205	0	205	205	0	205	205
Non-US adults	200	0	200	200	0	200	200
Children/adolescents (United States and outside the United States)	50	0	50	50	0	50	50
Pregnant women (HIV positive; United States and outside the United States)	60	0	60	60	0	60	60
HIV-1 group M subtypes (outside the United States)	75	0	75	75	0	75	75
HIV-1 p24 antigen/antibody (outside the United States)	50	0	50	50	0	50	50
Total	1,460	0	1,460	1,460	0	1,460	1,460
Individuals at high risk for HIV							
Adults (United States)	499	485	14	14	488	11	11
Children/adolescents (United States)	134	133	1	1	133	1	1
Pregnant women (United States and outside the United States)	125	89	36	36	96	29	29
First trimester	18	11	7	7	11	7	7
Second trimester	44	30	14	14	33	11	11
Third trimester	63	48	15	15	52	11	11
Total	758	707	51	51	717	41	41
Individuals at low risk for HIV							
Adults (United States)	6,050	5,965	91	85	5,960	92	90
Children/adolescents (United States)	591	589	3	2	591	0	0
Confirmed HIV-negative pregnant women (United States)	202	202	0	0	202	0	0
Total	6,843	6,756	94	87	6,753	92	90
HIV-2 endemic area cohort (outside the United States)	706	421	286	285	422	284	284
Healthy pregnant women (United States) ^b	290	290	0	0	290	0	0
HIV-1 p24 antigen positive, antibody negative, and Western blot negative/indeterminate (outside the United States)	27	1	26	NT	1	26	NT
HIV-1 group O cohort (outside the United States)	42	0	42	42	0	42	42

HIV, human immunodeficiency virus; IR, initially reactive; NR, nonreactive; NT, not tested; RR, repeatedly reactive.

^aAdults and HIV-negative pregnant women were 21 years or older; other pregnant women cohorts were 18 years or older; children/adolescents were aged 2 or more to less than 22 years. Although the inclusion criteria in the study protocol specified age 22 years or older for adult and pregnant women cohorts, some individuals aged 18 to 21 years were included in these cohorts under a waiver; however, most adults and pregnant women were 22 years or older.

^bHealthy pregnant women included confirmed HIV-negative pregnant women (n = 202) and the pregnant women subgroup of adults at low risk for HIV (self-reported as pregnant; n = 88).

49.99%-83.88%), and sensitivity was 100.00% (15/15; 95% CI, 78.20%-100.00%). Secondary analysis (against HIV-1, HIV-2, and p24 antigen individually) in high-risk pregnant women samples (United States, n = 1; outside the United States, n = 9) that were reactive on the Eleclys HIV combi PT assay but had a final diagnosis of HIV negative demonstrated that 8 of 10 samples were true positives. Recalculation of specificity and sensitivity yielded a specificity of 97.80% (89/91; 95% CI, 92.29%-99.73%) and sensitivity of 100.00% (34/34; 95% CI, 89.72%-100.00%) in the overall high-risk pregnant women cohort (United States and outside the United States), as well as a specificity of 95.65% (22/23; 95% CI, 78.05%-99.89%) and sensitivity of 100.00% (24/24; 95% CI, 85.75%-100.00%) in the non-US cohort.

Sensitivity of the Eleclys HIV combi PT assay for samples reactive for HIV-1 p24 antigen, antibody negative, and Western blot negative/indeterminate was 96.30% (95% CI, 81.03%-99.91%). All HIV-1 group M samples were reactive using the Eleclys HIV combi PT assay and reference assay (Supplementary Table 7). Sensitivity of the Eleclys HIV combi PT assay in the HIV-1 group O cohort (from Cameroon) was 100.00% (42/42; 95% CI, 91.59%-100.00%).

Clinical Specificity

Overall specificity of the Eleclys HIV combi PT assay in individuals at low risk for HIV (United States; n = 6,843, including adults, children/adolescents, and

confirmed HIV-negative pregnant women) was 99.94% (6,754/6,758; 95% CI, 99.85%-99.98%). In low-risk adults (excluding pregnant women), specificity was 99.97% (5,963/5,965; 95% CI, 99.88%-100.00%; [Table 2](#)).

Analytical Performance

Repeatability (within-run precision) and intermediate precision (within-laboratory precision) for eight human serum pools (HSPs) and five levels of controls are shown in [Table 3](#). Analysis of the HSPs on the Elecsys HIV combi PT assay produced mean COIs and CVs that were consistent with PreciControl controls. Reproducibility measurements evaluating between-run, between-day, between-lot, and between-site variations were also similar between HSPs and PreciControl controls. All precision and reproducibility tests with positive samples exhibited acceptable performance.

Seroconversion Detection Sensitivity

Twenty commercial seroconversion panels consisting of 140 bleeds were compared between the Elecsys HIV combi PT assay on the cobas e 602 analyzer and the Certificate of Analysis results from the reference assay where available (or compared with the reference assay result from clinical testing if not available). Equivalent performance was initially observed in 138 of 140 samples, but equivalent results were observed in all 140 samples after retesting in duplicate. All 20 panels demonstrated seroconversion from nonreactive to reactive.

Analytical Sensitivity/Specificity

Analytical sensitivity of the Elecsys HIV combi PT assay in an internal study (0.84 ± 0.11 IU/mL) was within

the expected range (≤ 2 IU/mL). A total of 293 samples from individuals with other viral diseases/medical conditions and confirmed HIV-negative pregnant women were tested for cross-reactivity with the Elecsys HIV combi PT assay; no interference was observed from any of the agents tested.

Potential Interfering Analytes, Endogenous Substances, and Therapeutic Drugs

The Elecsys HIV combi PT assay demonstrated an overall specificity of 100.00% (10/10; 95% CI, 69.15%-100.00%) for each virus tested (for hepatitis C, specificity was 100.00% [9/9; 95% CI, 66.37%-100.00%]). No false-positive results (neat samples) and no false-negative results (spiked samples) were observed.

No interference was observed with biotin up to 49 ng/mL, bilirubin up to 60 mg/dL, hemoglobin up to 500 mg/dL, intralipid up to 1,500 mg/dL, human serum albumin up to 10 g/dL, or rheumatoid factors up to 1,500 IU/mL.

No interference was observed with each of the 18 therapeutic drugs at the concentrations examined ([Supplementary Table 2](#)).

Discussion

Diagnostic assays for use in HIV screening must be highly sensitive and capable of detecting all subtypes of HIV. The ability to detect infection early in the disease course is also necessary to facilitate prompt treatment and prevent further transmission. The Elecsys HIV combi PT assay has been designed with a pretreatment step to enhance early detection and increase the assay sensitivity

Table 3
Repeatability, Precision, and Reproducibility of the Elecsys HIV combi PT Assay on the cobas e 602 Analyzer

Sample	Mean	Repeatability		Interrun		Interday		Intersite * Lot		Intersite		Interlot		Reproducibility	
		SD	CV, %	SD	CV, %	SD	CV, %	SD	CV, %	SD	CV, %	SD	CV, %	SD	CV, %
HSP															
06	2.030	0.087	4.30	0.018	0.88	0.045	2.22	0.022	1.08	0.024	1.18	0.058	2.87	0.120	5.91
07	51.200	2.010	3.93	0	0	0.489	0.95	0.666	1.30	0.639	1.25	1.270	2.48	2.590	5.07
08	1.430	0.049	3.46	0	0	0.022	1.56	0.200	1.37	0.011	0.77	0.058	4.07	0.083	5.78
09	38.200	1.300	3.42	0	0	0.368	0.96	0.378	0.99	0.563	1.47	0.890	2.34	1.760	4.61
10	2.250	0.157	6.97	0	0	0.029	1.29	0.033	1.46	0.027	1.19	0.066	2.94	0.177	7.90
11	58.700	1.970	3.35	0	0	0.227	0.39	0.756	1.29	0.656	1.12	2.310	3.94	3.210	5.46
12	4.390	0.181	4.13	0	0	0	0	0.046	1.05	0.067	1.52	0.216	4.93	0.294	6.69
13	0.082	0.014	17.59	0.011	12.95	0.007	8.90	0.009	10.94	0	0	0.021	25.94	0.030	36.73
PC															
HIV1	0.209	0.018	8.79	0.008	3.81	0.008	4.03	0.011	5.47	0	0	0.035	16.56	0.042	20.31
HIV2	4.830	0.168	3.48	0	0	0.035	0.72	0.048	0.99	0.035	0.73	0.165	3.41	0.245	5.08
HIV3	3.920	0.117	2.99	0.032	0.83	0.050	1.27	0.050	1.26	0	0	0.093	2.37	0.168	4.30
HIV4	5.010	0.127	2.54	0	0	0.050	1.00	0.054	1.07	0.042	0.84	0.286	5.71	0.324	6.47
HIV5	5.170	0.169	3.28	0	0	0.061	1.19	0.065	1.26	0.073	1.42	0.233	4.50	0.310	6.00

CV, coefficient of variation; HIV, human immunodeficiency virus; HSP, human serum pool; PC, PreciControl.

and specificity. In the current study, we demonstrate robust clinical and analytical performance of the Elecsys HIV combi PT assay under routine laboratory conditions, as well as good precision, and the assay reliably differentiated between HIV-positive and HIV-negative samples with high sensitivity and specificity relative to the final diagnosis. Importantly, the assay was well within regulatory authority criteria that require commercially available assays to detect both antigen and antibodies with a sensitivity for detection of p24 antigen of 2 IU/mL or less. Seroconversion detection sensitivity of the Elecsys HIV combi PT assay was similar to the FDA-approved reference assay, and no interference was observed from other viral disease states/medical conditions or commonly used drugs. These findings supported the FDA approval (June 2017) of the Elecsys HIV combi PT assay for diagnosing HIV-1 and/or HIV-2 infection (including acute or primary HIV-1 infection) in individuals 2 years or older and in pregnant women.

The current evaluation of the Elecsys HIV combi PT assay included almost 10,000 samples, the majority of which were US clinical samples, but non-US cohorts were also included. In this population, overall sensitivity was 100% and specificity was 99.94%, which is consistent with previous studies of the Elecsys HIV combi PT assay conducted in different geographical regions.^{7,8,13} A study conducted in 12 centers across Europe, Australia, and Thailand showed an overall specificity of 99.81% in routine samples and 99.88% in blood donor samples,⁷ while studies in Asia and South Korea have shown overall specificities of 99.86% and 99.50%, respectively.^{8,13} Together, these studies demonstrate consistently high sensitivity and specificity for the Elecsys HIV combi PT assay and therefore confirm its applicability across a broad range of geographical regions.

The predominance of different HIV subtypes in different regions is a product of the rapid evolution of HIV, which has led to considerable genetic diversity. This diversity is a known challenge for HIV assays, with previous studies reporting difficulties detecting certain subtypes, such as group O.^{14,15} Our study included cohorts from HIV-2 endemic regions, as well as samples with HIV-1 group O and M subtypes. The Elecsys HIV combi PT assay successfully detected known HIV-positive samples with high sensitivity regardless of HIV group or subtype and across different patient populations.

The Elecsys HIV combi PT assay showed excellent clinical performance and detection of seroconversion in comparison with the reference ARCHITECT HIV Ag/Ab combo assay. Furthermore, the high sensitivity observed in confirmed HIV-1–positive samples (100%) is equivalent or superior to the sensitivities previously determined

for other commercially available assays, including the ARCHITECT HIV Ag/Ab combo (99.94%), BioPlex 2200 (Bio-Rad; 100%), and Centaur HIV Ag/Ab Combo assays (Siemens Healthcare Diagnostics; 100%).¹⁶⁻¹⁸ The Elecsys HIV combi PT assay's specificity (99.94%) is also equivalent or superior to other HIV assays (98%-100%).^{7,16-22} The analytical sensitivity of the HIV combi PT assay (.84 IU/mL) in the present study is lower than previously reported (.90-1.05 IU/mL) and well within the sensitivity of 2 IU/mL or less specified by regulatory authorities. Furthermore, these data compare favorably with other fourth-generation assays (ARCHITECT HIV Ag/Ab combo, .94 IU/mL; Centaur HIV Ag/Ab combo, 1.89 IU/mL; AxSYM HIV Ag/Ab combo, 1.20 IU/mL)^{7,8} and demonstrate the suitability of the Elecsys HIV combi PT assay for use in HIV screening.

Early diagnosis of HIV in pregnant women is important so that antiretroviral therapy can be initiated as soon as possible to reduce the risk of vertical transmission, and the CDC recommends that all women are screened for HIV upon entering prenatal care.¹⁰ In samples from pregnant women, the Elecsys HIV combi PT assay demonstrated an overall specificity of 97.43%; in pregnant women at high risk for HIV infection, specificity and sensitivity were 89.90% and 100.00%, respectively. It should be noted that the high reactivity of non-US high-risk pregnant women samples was the major factor contributing to the lower specificity of the overall high-risk pregnant women cohort (specificity in the US cohort was 98.53%). A secondary analysis based on retesting with individual reagent components showed that only two of 10 samples were false positives, resulting in specificities of 97.80% and 95.65% in the overall and non-US cohorts, respectively. Our results suggest that the Elecsys HIV combi PT assay may improve accuracy of HIV diagnosis in pregnant women compared with the reference assay, as well as reduce emotional stress and unnecessary follow-up associated with false-positive results.²³

This study was designed to avoid biases in evaluation of clinical and analytical performance by obtaining samples from different collection sites and commercial vendors, as well as selecting samples for study testing based on clear inclusion and exclusion criteria. Analytical performance testing was conducted according to CLSI guidelines, and effects of multiple lots of reagents were evaluated in different final-user environments. Some of the population subgroup sample sizes were relatively small (ie, subgroups for confirmed HIV-positive pregnant women; pregnant women at high risk for HIV; confirmed HIV-1–positive children/adolescents; HIV-1 group O, HIV-1 group M subtypes; and antigen specimens), reflecting the rarity of some HIV subtypes and the

relatively low rate of HIV infection in pregnant women in the United States. As a result, the statistical power of some of the cohort-specific calculations of sensitivity and specificity was low.

In conclusion, our findings provide further evidence of the excellent clinical and analytical performance of the Elecsys HIV combi PT assay on the cobas e 602 analyzer for the detection of early HIV infection in high- and low-risk adults and children/adolescents, pregnant women, and HIV-1–positive individuals under routine laboratory conditions in the United States, and they supported the recent FDA approval.

Corresponding author: Denise Uettwiller-Geiger, PhD;
DGeiger@matherhospital.org

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