Elecsys HIV Duo

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REF		Σ		Rx Only	SYSTEM
08836973162	08836973502	10 x 300			**cobas pro** serology solution

English

For use in the USA only

System information

Short name	ACN (application code number)	Assay type	To be used for
HIVDUOB	12510	**cobas e** flow	HIV Duo
HIVDUOBR	12511	**cobas e** flow	HIV Duo duplicate repeat
HIVAGB[a)]	11511	HIV Antigen (HIV Ag) embedded application	HIV Duo **cobas e** flow
AHIVB[a)]	11510	Anti-HIV embedded application	HIV Duo **cobas e** flow

a) Embedded application within HIV Duo

Intended use

Elecsys HIV Duo is an in vitro immunoassay for the simultaneous qualitative detection and differentiation of HIV-1 p24 antigen and antibodies to HIV, HIV-1 (groups M and O) and HIV-2 in human serum and plasma. Elecsys HIV Duo is intended to screen individual human donors, including volunteer donors of whole blood, blood components and source plasma. The assay is also intended to be used to screen organ, tissue and cell donors, when donor samples are obtained while the donor's heart is still beating. It is not intended for use on cord blood specimens.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use with the **cobas pro** serology solution equipped with **cobas e** 801 analytical unit.

Summary

The human immunodeficiency virus (HIV), the causative agent of Acquired ImmunoDeficiency Syndrome (AIDS), belongs to the family of retroviruses. HIV can be transmitted through sexual contact, contaminated blood and blood products or from an HIV-infected mother to her child before, during and after birth.
Two types of HIV, called HIV-1 and HIV-2, have been identified to date.[1,2,3,4] HIV-1 can be divided into 4 distantly related groups: group M (for main), group N (for non-M, non-O), group O (for outlier) and group P (Plantier).[5,6,7] Based on their genetic relationship, 10 different subtypes (A to D, F to H, J, K and L) as well as several circulating recombinant forms (CRFs) have been identified within HIV-1 group M.[8,9] The large majority of HIV-1 infections are caused by viruses belonging to group M, while geographical distribution of subtypes and CRFs within this group varies strongly.[10] Due to differences in the sequence of immunodominant epitopes, especially in the envelope proteins of HIV-1 group M, HIV-1 group O and HIV-2, specific antigens are necessary to avoid failure in the detection of an HIV infection by immunoassays.[11,12]

HIV p24 antigen in blood specimens of recently infected patients can be detected as early as 2-3 weeks after infection.[13,14] Anti-HIV antibodies are detectable in serum from around 4 weeks post infection.[13,15] The combined detection of HIV p24 antigen and anti-HIV antibodies in 4th generation HIV screening assays leads to improved sensitivity compared to traditional anti-HIV assays.[16,17]

With the Elecsys HIV Duo assay, HIV-1 p24 antigen (HIV Ag), as well as antibodies to HIV-1 and HIV-2 (anti-HIV) can be detected in parallel with two separate determinations. On the basis of these determinations, the Elecsys HIV Duo main result is subsequently calculated automatically by the analytical unit. The subresults HIV Ag and anti-HIV can be used as an aid in the selection of the confirmation algorithm for reactive samples. The Elecsys HIV Duo assay uses monoclonal antibodies to detect the HIV Ag and recombinant antigens derived from the Env- and Pol-region of HIV-1 (including group O) and HIV-2 to detect anti-HIV antibodies.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: For HIV Ag detection (HIVAGB), 30 µL of sample react with biotinylated monoclonal anti-p24 antibodies and ruthenylated[b)] monoclonal anti-p24 antibodies, to form a sandwich complex. For anti-HIV detection (AHIVB), 30 µL of sample react with biotinylated HIV-specific recombinant antigens/peptides and ruthenylated[b)] HIV-specific recombinant antigens/peptides, to form a sandwich complex. The incubations are performed in parallel in separate vessels.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined automatically by the software by comparing the electrochemiluminescence signal obtained from the sample with the cutoff value obtained by HIV Ag embedded and anti-HIV embedded calibrations. The Elecsys HIV Duo result is calculated automatically based on signal to cutoff ratios (cutoff index, COI) from HIV Ag and anti-HIV.

b) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The **cobas e** pack HIV Ag (M, R1, R2) is labeled as HIVAGB.

The **cobas e** pack Anti-HIV (M, R1, R2) is labeled as AHIVB.

HIVAGB

M Streptavidin-coated microparticles, 1 bottle, 14.1 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-HIV p24-Ab~biotin, 1 bottle, 14.8 mL: Biotinylated monoclonal anti-HIV p24 antibodies (mouse) approximately 0.75 mg/L; MES[c)] buffer 50 mmol/L, pH 6.5; preservative.

R2 Anti-HIV p24-Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 14.8 mL: Monoclonal anti-p24 antibodies (mouse) labeled with ruthenium complex approximately 0.75 mg/L; MES[c)] buffer 50 mmol/L, pH 6.5; preservative.

c) MES = 2-morpholino-ethane sulfonic acid

HIVDUOB Cal1 Non-reactive calibrator 1 (lyophilized), 2 vials each for 1.0 mL: Human serum, non-reactive for anti-HIV-1 and anti-HIV-2; preservative.

HIVDUOB Cal2 Reactive calibrator 2 (lyophilized), 2 vials each for 1.0 mL: HIV p24 antigen (E. coli, rDNA) in human serum, non-reactive for anti-HIV-1 and anti-HIV-2; preservative.

AHIVB

M Streptavidin-coated microparticles, 1 bottle, 14.1 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 HIV-1/2-specific recombinant antigens (E. coli)~biotin, HIV-1/2-specific synthetic peptides~biotin, 1 bottle, 14.8 mL: Biotinylated HIV-1/2-specific recombinant antigens (E. coli) and biotinylated HIV-1/2 specific synthetic peptides approximately 0.63 mg/L; TES[d)] buffer 40 mmol/L, pH 7.3; preservative.

R2 HIV-1/2-specific recombinant antigens (E. coli)~$Ru(bpy)_3^{2+}$, HIV-1/2-specific synthetic peptides~$Ru(bpy)_3^{2+}$, 1 bottle, 14.8 mL: HIV-1/2-specific recombinant antigens (E. coli) and HIV-1/2-specific synthetic peptides labeled with ruthenium complex approximately 1.22 mg/L; TES[d)] buffer 40 mmol/L, pH 7.3; preservative.

d) TES = 2-[[1,3-dihydroxy-2-(hydroxymethyl)propane-2-yl]amino]ethanesulfonic acid

IVD

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HIVDUOB Cal3 Non-reactive calibrator 3 (lyophilized), 2 vials each for 1.0 mL: Human serum, non-reactive for anti-HIV-1 and anti-HIV-2; preservative.

HIVDUOB Cal4 Reactive calibrator 4 (lyophilized), 2 vials each for 1.0 mL: Anti-HIV-1 reactive human serum (inactivated) in human serum non-reactive for anti-HIV-1 and anti-HIV-2; preservative.

Precautions and warnings

For in vitro diagnostic use.

This test is not intended for use as an aid in diagnosis of HIV-1/HIV-2 infection.

Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317 May cause an allergic skin reaction.

H412 Harmful to aquatic life with long lasting effects.

Prevention:

P261 Avoid breathing mist or vapours.

P273 Avoid release to the environment.

P280 Wear protective gloves.

Response:

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: 1-800-428-2336

All human material should be considered potentially infectious.

The non-reactive calibrators (HIVDUOB Cal1 and HIVDUOB Cal3) as well as the HIV Ag reactive calibrator (HIVDUOB Cal2) have been prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods use assays that have been approved by the FDA or that are in compliance with the legal rules applicable to placing in vitro diagnostic medical devices for human use on the market in the European Union.

The serum containing anti-HIV-1 (HIVDUOB Cal4) was inactivated using ß-propiolactone and UV-radiation.

However, as no inactivation or testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a donor specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[18,19]

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents (M, R1, R2) in the kit are ready-for-use and are supplied in **cobas e** packs.

Calibrators

Carefully dissolve the contents of one vial by adding exactly 1.0 mL of distilled or deionized water and allow to stand closed for 15 minutes to reconstitute. Mix carefully, avoiding foam formation.

Transfer the reconstituted calibrators into the supplied empty labeled snap-cap vials.

Perform **only one** calibration procedure per vial.

All information required for correct operation is available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability of the **cobas e** pack:

unopened at 2-8 °C	up to the stated expiration date
on the **cobas e** 801 analytical unit	16 weeks

Stability of the calibrators:

unopened at 2-8 °C	up to the stated expiration date
reconstituted at 2-8 °C	3 days
on the **cobas e** 801 analytical unit at 20-25 °C	use only once, stable onboard for up to 5 hours

Store calibrators **upright** in order to prevent the calibrator solution from adhering to the lid of the vials.

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Serum and Li-heparin, K_2-EDTA, K_3-EDTA, CPD and Na-citrate plasma collected using standard sampling tubes.

Serum and Li-heparin and K_2-EDTA plasma collected in tubes containing separating gel.

Stable on-the-clot for 7 days at 15-30 °C and 14 days at 2-8 °C. Do not freeze samples on-the-clot.
Samples off-the-clot are stable for 7 days at 20-25 °C, 14 days at 2-8 °C and 1 month at -20 °C (± 5 °C). Samples off-the-clot may be frozen up to 4 times.

Specimens collected by plasmapheresis, which have not been frozen, do not require centrifugation. All other whole-blood samples and samples containing precipitates need to be centrifuged before performing the assay for 10 to 15 minutes at 2000 to 4000 RCF (relative centrifugal force = x g).

The sample types listed were tested with a selection of sample collection tubes or systems that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube/collection system manufacturer.

Do not use pools of samples.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

The performance of Elecsys HIV Duo has not been established with cadaveric samples or body fluids other than serum and plasma.

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet.

Materials provided

See “Reagents – working solutions” section for reagents.

- 8 empty labeled snap-cap vials

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Materials required (but not provided)

- REF 06924107162, PreciControl HIV Gen II, for 6 x 2.0 mL
- REF 06924115162, PreciControl HIV; HIV-2+GrpO, for 4 x 2.0 mL
- REF 09367101190, PreciControl Release HIV Gen II, for 6 x 2.0 mL
- General laboratory equipment
- The **cobas pro** serology solution is a combination of the **cobas pro** serology controller, **cobas pro** integrated solutions (**cobas e** 801 analytical units only) and applicable licensed or cleared donor screening assays.
- Distilled or deionized water

Additional materials for **cobas e** 801 analytical unit:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500160, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analytical unit concerned. Refer to the appropriate user guide for analytical unit specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibrators:

Place the reconstituted calibrators in the sample zone.

Read in all the information necessary for calibrating the assay.

Calibration

Traceability:
HIVAGB: This method has been standardized against the WHO International Standard HIV-1 p24 Antigen, NIBSC (National Institute for Biological Standards and Control) code 90/636.

AHIVB: No internationally accepted standard for anti-HIV-1 and anti-HIV-2 exists.

Calibration frequency: Calibration must be performed once per reagent lot using HIVDUOB Cal1, HIVDUOB Cal2, HIVDUOB Cal3, HIVDUOB Cal4 and fresh reagent (i.e. not more than 24 hours since the **cobas e** pack was registered on the analytical unit).

Recalibration is required as follows:

- after 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analytical unit
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl HIV Gen II and PreciControl HIV; HIV-2+GrpO.

Controls for the various concentration ranges must be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, and following each calibration.

PreciControl HIV Gen II and PreciControl HIV; HIV-2+GrpO values must be within the ranges specified in the control value sheet. When the assay control values are within range, sample results are generated, and a valid release control result is required to release test results. If an assay control value is not within range, sample results are not generated for in-process or scheduled samples. For troubleshooting information, refer to User Assistance **cobas pro** serology solution or contact US Customer Technical Support.

Release control

For release control, use PreciControl Release HIV Gen II.

Result validation is based on test result batches that are concluded by release control measurements. A release control result within defined limits is required to validate a batch of previously measured test results utilizing the **cobas pro** serology controller software. Initial reactive results will not be invalidated by a failed release control and must be retested in duplicate. Repeatedly reactive results will not be invalidated by a failed release control and stay reactive. Other results rendered invalid due to a failed release control result must be retested after resolving the cause for the failed control measurement.

For a valid batch of sample results, the release control is tested at user-defined intervals with a maximum span of every 300 samples or 350 determinations within 24 hours from the PreciControl and must be tested in order to release the test results. Reactive results will not be invalidated. The release control must meet specifications defined in the PreciControl Release HIV Gen II value sheet in order to validate the system functionality and release test results. For troubleshooting information, refer to User Assistance **cobas pro** serology solution or contact US Customer Technical Support.

Calculation

The analytical unit automatically calculates the cutoff based on the measurement of HIVDUOB Cal1 and HIVDUOB Cal2 for the HIVAGB module, and HIVDUOB Cal3 and HIVDUOB Cal4 for the AHIVB module.

The result of a sample is given either as reactive or non-reactive as well as in the form of a cutoff index (signal sample/cutoff).

The following formula is used to calculate the main Elecsys HIV Duo result based on the HIVAGB and AHIVB subresults:

$$\text{HIVDUOB (COI)} = \sqrt{(\text{HIVAGB [COI]})^2 + (\text{AHIVB [COI]})^2}$$

Interpretation of the results (HIVDUOB or HIVDUOBR)

Initial main result

Numeric result	Result	Interpretation
COI < 1.00	Non-reactive	Non-reactive for HIV-1 Ag and non-reactive for anti-HIV-1/2 antibodies. No further testing needed.
COI ≥ 1.00	Reactive	Reactive in the Elecsys HIV Duo assay. All initially reactive samples should be retested in duplicate with the Elecsys HIV Duo assay. Redetermination of samples with an initial COI ≥ 1.00 can be performed automatically (see section **cobas e** flow).

Subresult HIVAGB

Numeric result	Result	Interpretation
COI < 1.00	Non-reactive	Non-reactive for HIV-1 p24 antigen.
COI ≥ 1.00	Reactive	Reactive in the HIV-1 Ag module.

Subresult AHIVB

Numeric result	Result	Interpretation
COI < 1.00	Non-reactive	Non-reactive for anti-HIV-1/2 antibodies.
COI ≥ 1.00	Reactive	Reactive in the anti-HIV module.

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Final result

Numeric result	Final result	Interpretation / further steps
Both of the duplicate retests have a COI < 1.00	Non-reactive	Non-reactive for HIV-1 Ag and non-reactive for anti-HIV-1/2 antibodies. No further testing needed.
One or both of the duplicate retests have a COI ≥ 1.00	Repeatedly reactive	Repeatedly reactive samples must be confirmed according to supplementary algorithms. The subresults for either HIVAGB or AHIVB can be used as an aid in the selection of the confirmation algorithm for reactive samples.

Please note: In cases where the HIVAGB and AHIVB results are both non-reactive with high negative results near the cutoff, the formula may result in an Elecsys HIV Duo result with a COI ≥ 1.00 and reported as reactive. These results will be flagged as "Antigen test negative, Antibody test negative" and considered as reactive and should be retested in duplicate with the Elecsys HIV Duo assay.

cobas e flow

A **cobas e** flow is a procedure programmed into the system to enable a fully automated sequence of measurements and the calculation of assay combinations to perform decision algorithms.
The HIVDUOB **cobas e** flow is required to enable simultaneous separate measurement of HIVAGB and AHIVB with subsequent calculation of the main result.
A second HIV **cobas e** flow is available to perform a repetition of measurements in duplicate automatically for samples with an initial cutoff index ≥ 1.00 (short name HIVDUOBR). Both subresults and the overall result message will be reported.

Limitations of test

A non-reactive test result does not rule out the possibility of an infection with HIV. Serum or plasma samples from the very early (pre-seroconversion) phase or the late phase of HIV infection can occasionally yield non-reactive findings. Yet unknown HIV variants can also lead to non-reactive HIV findings.

False reactive results may be observed due to non-specific interactions.

The detection of HIV-1 p24 antigen or HIV-1/HIV-2 antibodies is not a diagnosis of HIV. It is recommended that repeatedly reactive specimens be confirmed by supplemental testing. Individuals who are repeatedly reactive should be referred for medical evaluation which may include additional testing.

The performance of the Elecsys HIV Duo assay has not been established with cord blood, neonatal specimens, cadaveric specimens, heat-inactivated specimens, or body fluids other than serum and plasma.

Specific performance data

Representative performance data is given below. Results obtained in individual laboratories may differ.

Reproducibility

A study was performed based on guidance from CLSI EP05-A3 (n = 270). Testing was conducted at 3 external sites using 3 lots of the Elecsys HIV Duo assay and 1 lot each of PreciControl HIV Gen II, PreciControl HIV; HIV-2+GrpO and PreciControl Release HIV Gen II. Panel members and controls were tested in 2 runs per day for 5 days with 3 sample replicates per run. The precision and reproducibility for the Elecsys HIV Duo assay are presented in the following tables.

Overall repeatability and reproducibility for Elecsys HIV Duo (Main result)

Sample	Mean (COI)	Repeatability SD (COI)	Repeatability % CV	Between run SD (COI)	Between run % CV
HSP 01 e)	2.71	0.036	1.32	0.017	0.630
HSP 02	22.3	0.313	1.41	0.185	0.832
HSP 03	2.61	0.035	1.34	0.027	1.02
HSP 04	22.4	0.269	1.20	0.176	0.784
HSP 05	2.63	0.041	1.56	0.022	0.819
HSP 06	3.09	0.048	1.54	0.022	0.704
HSP 07	26.4	0.353	1.34	0.191	0.725
PC HIV1 B f)	0.185	0.021	11.6	0.002	0.849
PC HIV2 B	3.62	0.058	1.60	0.047	1.31
PC HIV3 B	9.00	0.103	1.14	0.118	1.31
PC HIV4 B	4.12	0.071	1.74	0.046	1.12
PC HIV5 B	5.96	0.114	1.92	0.084	1.40

e) HSP = human specimens

f) PC = PreciControl

Overall repeatability and reproducibility for Elecsys HIV Duo (Main result)

Sample	Mean (COI)	Between day SD (COI)	Between day % CV	Intermediate precision SD (COI)	Intermediate precision % CV
HSP 01	2.71	0.041	1.53	0.057	2.12
HSP 02	22.3	0.286	1.29	0.463	2.08
HSP 03	2.61	0.036	1.39	0.057	2.19
HSP 04	22.4	0.441	1.97	0.546	2.44
HSP 05	2.63	0.046	1.75	0.065	2.48
HSP 06	3.09	0.039	1.27	0.066	2.12
HSP 07	26.4	0.402	1.52	0.568	2.15
PC HIV1 B	0.185	0.008	4.20	0.023	12.3
PC HIV2 B	3.62	0.047	1.30	0.088	2.44
PC HIV3 B	9.00	0.120	1.33	0.197	2.19
PC HIV4 B	4.12	0.064	1.56	0.107	2.59
PC HIV5 B	5.96	0.062	1.05	0.155	2.60

Overall repeatability and reproducibility for Elecsys HIV Duo (Main result)

Sample	Mean (COI)	Between site SD (COI)	Between site % CV	Between lot SD (COI)	Between lot % CV
HSP 01	2.71	0.000	0.000	0.055	2.03
HSP 02	22.3	0.168	0.753	0.747	3.36
HSP 03	2.61	0.006	0.244	0.187	7.18
HSP 04	22.4	0.000	0.000	0.368	1.64
HSP 05	2.63	0.000	0.000	0.052	1.97
HSP 06	3.09	0.041	1.34	0.103	3.33
HSP 07	26.4	0.260	0.986	0.802	3.04
PC HIV1 B	0.185	0.004	2.35	0.017	8.97
PC HIV2 B	3.62	0.000	0.000	0.099	2.74
PC HIV3 B	9.00	0.062	0.688	0.246	2.73
PC HIV4 B	4.12	0.002	0.037	0.217	5.28
PC HIV5 B	5.96	0.078	1.30	0.129	2.16

Overall repeatability and reproducibility for Elecsys HIV Duo (Main result)

Sample	Mean (COI)	Reproducibility SD (COI)	Reproducibility % CV
HSP 01	2.71	0.080	2.94
HSP 02	22.3	0.895	4.02
HSP 03	2.61	0.196	7.51
HSP 04	22.4	0.658	2.94
HSP 05	2.63	0.083	3.17
HSP 06	3.09	0.129	4.17
HSP 07	26.4	1.02	3.86

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Sample	Mean (COI)	Reproducibility SD (COI)	Reproducibility % CV
PC HIV1 B	0.185	0.028	15.4
PC HIV2 B	3.62	0.133	3.67
PC HIV3 B	9.00	0.321	3.56
PC HIV4 B	4.12	0.242	5.88
PC HIV5 B	5.96	0.216	3.62

Overall repeatability and reproducibility for Elecsys HIV Duo (HIVAGB module)

Sample	Mean (COI)	Repeatability SD (COI)	Repeatability % CV	Between run SD (COI)	Between run % CV
HSP 01	0.160	0.010	6.10	0.001	0.400
HSP 02	0.160	0.008	5.25	0.003	2.06
HSP 03	0.158	0.009	5.85	0.0002	1.20
HSP 04	0.160	0.009	5.49	0.001	0.871
HSP 05	0.160	0.009	5.53	0.003	1.88
HSP 06	3.09	0.047	1.53	0.023	0.733
HSP 07	26.4	0.353	1.34	0.191	0.725
PC HIV1 B	0.169	0.010	6.06	0.000	0.000
PC HIV2 B	0.165	0.011	6.62	0.000	0.000
PC HIV3 B	9.00	0.103	1.14	0.118	1.31
PC HIV4 B	0.165	0.010	5.78	0.000	0.000
PC HIV5 B	0.167	0.009	5.59	0.002	1.50

Overall repeatability and reproducibility for Elecsys HIV Duo (HIVAGB module)

Sample	Mean (COI)	Between day SD (COI)	Between day % CV	Intermediate precision SD (COI)	Intermediate precision % CV
HSP 01	0.160	0.006	3.55	0.011	7.07
HSP 02	0.160	0.007	4.66	0.012	7.32
HSP 03	0.158	0.007	4.52	0.012	7.49
HSP 04	0.160	0.007	4.54	0.011	7.18
HSP 05	0.160	0.007	4.31	0.012	7.26
HSP 06	3.09	0.040	1.28	0.066	2.13
HSP 07	26.4	0.402	1.52	0.568	2.15
PC HIV1 B	0.169	0.007	4.40	0.013	7.49
PC HIV2 B	0.165	0.006	3.92	0.013	7.69
PC HIV3 B	9.00	0.120	1.33	0.197	2.19
PC HIV4 B	0.165	0.006	3.88	0.011	6.96
PC HIV5 B	0.167	0.007	4.10	0.012	7.09

Overall repeatability and reproducibility for Elecsys HIV Duo (HIVAGB module)

Sample	Mean (COI)	Between site SD (COI)	Between site % CV	Between lot SD (COI)	Between lot % CV
HSP 01	0.160	0.004	2.65	0.014	8.48
HSP 02	0.160	0.006	4.03	0.014	8.84
HSP 03	0.158	0.005	3.40	0.013	8.37
HSP 04	0.160	0.005	3.14	0.014	8.81
HSP 05	0.160	0.004	2.70	0.014	8.89
HSP 06	3.09	0.041	1.34	0.103	3.33
HSP 07	26.4	0.260	0.986	0.802	3.04

Sample	Mean (COI)	Between site SD (COI)	Between site % CV	Between lot SD (COI)	Between lot % CV
PC HIV1 B	0.169	0.007	4.10	0.016	9.66
PC HIV2 B	0.165	0.005	3.18	0.017	10.5
PC HIV3 B	9.00	0.062	0.688	0.246	2.73
PC HIV4 B	0.165	0.007	4.02	0.017	10.3
PC HIV5 B	0.167	0.005	2.74	0.018	10.5

Overall repeatability and reproducibility for Elecsys HIV Duo (HIVAGB module)

Sample	Mean (COI)	Reproducibility SD (COI)	Reproducibility % CV
HSP 01	0.160	0.018	11.4
HSP 02	0.160	0.019	12.2
HSP 03	0.158	0.019	11.7
HSP 04	0.160	0.019	11.8
HSP 05	0.160	0.019	11.8
HSP 06	3.09	0.129	4.17
HSP 07	26.4	1.02	3.86
PC HIV1 B	0.169	0.022	12.9
PC HIV2 B	0.165	0.022	13.4
PC HIV3 B	9.00	0.321	3.56
PC HIV4 B	0.165	0.022	13.1
PC HIV5 B	0.167	0.022	13.0

Overall repeatability and reproducibility for Elecsys HIV Duo (AHIVB module)

Sample	Mean (COI)	Repeatability SD (COI)	Repeatability % CV	Between run SD (COI)	Between run % CV
HSP 01	2.70	0.036	1.33	0.017	0.616
HSP 02	22.3	0.314	1.41	0.184	0.829
HSP 03	2.60	0.035	1.34	0.026	1.02
HSP 04	22.4	0.270	1.21	0.175	0.782
HSP 05	2.62	0.041	1.58	0.021	0.787
HSP 06	0.073	0.004	5.01	0.000	0.541
HSP 07	0.079	0.004	4.87	0.002	2..06
PC HIV1 B	0.072	0.024	33.9	0.002	3.28
PC HIV2 B	3.61	0.059	1.62	0.047	1.29
PC HIV3 B	0.069	0.003	4.88	0.001	1.71
PC HIV4 B	4.11	0.071	1.73	0.047	1.14
PC HIV5 B	5.96	0.114	1.92	0.084	1.41

Overall repeatability and reproducibility for Elecsys HIV Duo (AHIVB module)

Sample	Mean (COI)	Between day SD (COI)	Between day % CV	Intermediate precision SD (COI)	Intermediate precision % CV
HSP 01	2.70	0.041	1.53	0.057	2.11
HSP 02	22.3	0.287	1.29	0.464	2.08
HSP 03	2.60	0.036	1.39	0.057	2.18
HSP 04	22.4	0.442	1.97	0.547	2.44
HSP 05	2.62	0.046	1.76	0.065	2.49
HSP 06	0.073	0.002	2.45	0.004	5.60
HSP 07	0.079	0.002	2.68	0.005	5.93

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Sample	Mean (COI)	Between day SD (COI)	Between day % CV	Intermediate precision SD (COI)	Intermediate precision % CV
PC HIV1 B	0.072	0.000	0.000	0.025	34.0
PC HIV2 B	3.61	0.047	1.31	0.089	2.45
PC HIV3 B	0.069	0.001	2..05	0.004	5.56
PC HIV4 B	4.11	0.064	1.56	0.107	2.59
PC HIV5 B	5.96	0.063	1.05	0.155	2.61

Overall repeatability and reproducibility for Elecsys HIV Duo (AHIVB module)

Sample	Mean (COI)	Between site SD (COI)	Between site % CV	Between lot SD (COI)	Between lot % CV
HSP 01	2.70	0.000	0.000	0.056	2.07
HSP 02	22.3	0.167	0.752	0.748	3.36
HSP 03	2.60	0.007	0.267	0.189	7.25
HSP 04	22.4	0.000	0.000	0.369	1.65
HSP 05	2.62	0.000	0.000	0.053	2.01
HSP 06	0.073	0.002	2.98	0.010	13.5
HSP 07	0.079	0.004	4.61	0.010	12.9
PC HIV1 B	0.072	0.002	2.54	0.005	7.36
PC HIV2 B	3.61	0.000	0.000	0.099	2.75
PC HIV3 B	0.069	0.003	3.84	0.009	12.6
PC HIV4 B	4.11	0.000	0.000	0.218	5.30
PC HIV5 B	5.96	0.078	1.31	0.129	2.17

Overall repeatability and reproducibility for Elecsys HIV Duo (AHIVAB module)

Sample	Mean (COI)	Reproducibility SD (COI)	Reproducibility % CV
HSP 01	2.70	0.080	2.96
HSP 02	22.3	0.896	4.03
HSP 03	2.60	0.197	7.57
HSP 04	22.4	0.659	2.95
HSP 05	2.62	0.084	3.20
HSP 06	0.73	0.011	14.9
HSP 07	0.079	0.012	14.9
PC HIV1 B	0.072	0.025	34.9
PC HIV2 B	3.61	0.133	3.69
PC HIV3 B	0.069	0.010	14.3
PC HIV4 B	4.11	0.243	5.90
PC HIV5 B	5.96	0.216	3.63

Results: The precision and reproducibility of the Elecsys HIV Duo assay demonstrated minor variability from run to run, day to day and between reagent lots.

Analytical specificity

The effect of the following endogenous substances on assay performance were tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 753 µmol/L or ≤ 44 mg/dL
Hemoglobin	≤ 0.311 mmol/L or ≤ 500 mg/dL
Intralipid	≤ 2000 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Albumin	≤ 7.0 g/dL

Additionally, naturally elevated samples for bilirubin, rheumatoid factor, triglycerides (lipemic), hemoglobin and albumin were tested; no false reactive results were found.

No false non-reactive result due to high-dose hook effect was found with the Elecsys HIV Duo assay.

In rare cases, interference due to extremely high titers of antibodies to immunological components, streptavidin or ruthenium can occur and these effects are minimized by assay formulation and design.

Clinical specificity

A total of 7071 fresh serum specimens and 7098 fresh plasma specimens from volunteer blood donors and 2999 plasmapheresis samples were collected at 4 donor centers. The specificities based on this study were 99.92 % (95 % confidence interval (CI) 99.81 %, 99.96 %) for the serum specimens, 99.89 % (95 % CI 99.78 %, 99.94 %) for the plasma specimens, and 100 % (95 % CI 99.87 %, 100 %) for the plasmapheresis samples.

Specificity of Elecsys HIV Duo

Specimen category	Number tested	IR g)	RR h)	Number positive by supplemental testing	Specificity (%) (95 % CI)
Volunteer blood donors - serum	7071	6 (0.08 %)	6 (0.08 %)	0 (0.00 %)	99.92 % (99.81 %, 99.96 %)
Volunteer blood donors - plasma	7098	8 (0.11 %)	8 (0.11 %)	0 (0.00 %)	99.89 % (99.78 %, 99.94 %)
Plasmapheresis donors - plasma	2999	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)	100 % (99.87 %, 100 %)
Total donors	17168	14 (0.08 %)	14 i) (0.08 %)	0 (0.00 %)	99.92 % (99.86 %, 99.95 %)

g) IR = initially reactive

h) RR = repeatedly reactive

i) Repeatedly reactive specimens were further tested using the following supplemental assays: (HIV1) Group M RNA, HIV1 Group O RNA, Human Immunodeficiency Virus Type 2 (HIV2) RNA qualitative immunochromatographic HIV1/HIV2 antibody assay. 14 specimens were repeatedly reactive and 13 of those were confirmed negative. 1 specimen was inconclusive due to a supplemental test system flag; however, this sample was included for specificity calculation based on non-reactive results with the comparator assay and NAT. The final status was determined to be negative, for a total of 14 confirmed negative.

Specificity based on assumed zero prevalence of antigen/antibody to HIV in blood and plasmapheresis donors was estimated in this study to be 99.92 % (17154/17168) with a 95 % confidence interval of 99.86 %, 99.95 %.

Clinical sensitivity

A total of 1977 HIV confirmed-reactive specimens from the categories shown in the table below were tested using the Elecsys HIV Duo assay at 3 clinical sites. Repeatedly reactive specimens were confirmed with an FDA-approved HIV-Ag-Ab assay and/or a reactive HIV NAT against the final HIV status. Sensitivity was estimated to be 100 % (1977/1977) with a 95 % confidence interval of 99.81 % to 100 %.

Sensitivity of Elecsys HIV Duo

Specimen category	Number tested	Number positive	Number RR (% of RR)	Number RR that were positive (% of RR)	Sensitivity (%) (95 % CI)
All samples known to be positive for antibodies to HIV-1	1409	1409	1409 (100 %)	1409 (100 %)	100 % (99.74 to 100 %)
All samples known to be positive for antibodies to HIV-2	200	200	200 (100 %)	200 (100 %)	100 % (98.17 to 100 %)
HIV-1 antigen positive / antibody negative	52	52	52 (100 %)	52 j) (100 %)	100 % (93.15 to 100 %)
HIV-1 viral lysates (Group M subtypes)	90	90	90 (100 %)	90 (100 %)	100 % (95.98 to 100 %)

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Specimen category	Number tested	Number positive	Number RR (% of RR)	Number RR that were positive (% of RR)	Sensitivity (%) (95 % CI)
HIV-1 Group O	50	50	50 (100 %)	50 j) (100 %)	100 % (92.89 to 100 %)
Individuals at high risk of HIV-1/2 infection	1410	162	168 (11.91 %)	162 (96.43 %)	100 % (97.75 to 100 %)
Individuals at low risk of HIV-1/2 infection	6910	14	21 (0.30 %)	14 (66.66 %)	100 % (76.84 to 100 %)
Total	10121	1977	1990 (19.66 %)	1977 (99.34 %)	100 % (99.81 to 100 %)

j) One sample for this specimen category QNS for repeat testing so the respective certificate of analysis was used to provide objective evidence of HIV infection.

Analytical sensitivity

Analytical sensitivity of Elecsys HIV Duo for detection of HIV-1 p24 antigen was evaluated using the WHO International Standard HIV-1 p24 antigen, NIBSC code 90/636. HIV-1 p24 antigen was diluted with HIV negative serum and measured with Elecsys HIV Duo. Seven dilution steps of each standard were prepared and measured. Sensitivity was calculated using the mean of 3 lots tested by reading off the concentration at the cutoff from the HIV-Ag reference standard curve. The analytical antigen sensitivity of Elecsys HIV Duo as measured by WHO International Standard HIV-1 p24 antigen, NIBSC code 90/636 was shown to be ≤ 1 IU/mL. Using 3 reagent lots of Elecsys HIV Duo, results were 0.393, 0.395 and 0.389 IU/mL (average 0.392 IU/mL).

Group and subtype detection

A total of 58 viral lysates, positive for different HIV-1 subtypes, CRFs, and HIV-2 in HIV-negative serum as well as HIV-1 antibody positive human serum or plasma, were tested and detected with Elecsys HIV Duo. To assess HIV-1 p24 antigen and HIV-1 antibody reactivity of HIV-1 group N and P, a single dilution of viral lysate positive for different HIV-1 Group N and Group P in HIV negative serum as well as HIV-1 antibody positive human serum or plasma was tested and detected with Elecsys HIV Duo.

Other specimen conditions or disease states

100 samples containing potentially interfering factors were tested with the Elecsys HIV Duo assay comprising specimens:

- containing antibodies against HAV, HBV, HCV, HTLV-I/II, CMV, EBV, HSV-1/2, Rubella, Rotavirus, Smallpox, VZV
- containing autoantibodies (ANA) and elevated titers of rheumatoid factor
- containing antibodies against *Candida* sp., *Escherichia coli*, *Plasmodium falciparum/vivax*, *Mycobacterium tuberculosis*, *Chlamydia trachomatis*, *Treponema pallidum* (syphilis)
- after vaccination against HAV, HBV and influenza
- from patients with monoclonal gammopathy and multiple myeloma/lymphoma, common cold, Graves' disease, Crohn's disease
- from pregnant women 1st, 2nd and 3rd trimester, multiparous pregnancies

Testing was conducted with neat specimens and aliquots individually spiked with HIV-1 antibody, HIV-2 antibody and HIV-1 p24 antigen. Results showed no interference from the above agents.

Seroconversion panels

Seroconversion sensitivity of the Elecsys HIV Duo assay was shown by testing 50 commercially available seroconversion panels comparing Elecsys HIV Duo results to the FDA-approved product. Representative results from 5 panels are summarized in the table below.

Elecsys HIV Duo assay reactivity in seroconversion panels

Panel/Donor	Days since first bleed	Elecsys HIV Duo Lot 1	Elecsys HIV Duo Lot 2	Elecsys HIV Duo Lot 3	FDA-approved product
0600-271	0	0.171	0.161	0.169	0.100
0600-271	3	0.436	0.425	0.387	0.600
0600-271	7	32.4	33.7	24.2	39.7
0600-271	10	20.0	19.9	14.7	25.2
0600-271	14	68.8	69.5	48.1	64.9
0600-271	18	11.8	11.3	8.63	13.1
0600-271	21	12.8	11.9	9.46	11.2
0600-271	25	14.9	13.6	11.1	19.0
9011	0	0.188	0.175	0.170	0.090
9011	4	0.169	0.176	0.173	0.110
9011	9	0.166	0.185	0.161	0.070
9011	11	0.181	0.180	0.171	0.230
9011	16	0.171	0.183	0.174	0.160
9011	18	0.162	0.182	0.181	0.110
9011	23	0.162	0.161	0.170	0.110
9011	25	0.178	0.153	0.157	0.080
9011	30	0.386	0.380	0.387	0.210
9011	38	7.05	7.38	7.01	3.78
9011	40	10.5	11.0	10.3	5.12
9012	0	0.165	0.165	0.176	0.100
9012	2	0.171	0.190	0.178	0.070
9012	7	0.181	0.186	0.168	0.080
9012	9	0.188	0.174	0.176	0.110
9012	14	0.874	0.897	0.861	0.370
9012	16	2.99	3.11	2.95	1.02
9012	21	58.0	60.5	56.3	15.2
9012	23	138	143	135	50.0
PRB961	0	0.187	0.159	0.167	0.100
PRB961	5	0.176	0.176	0.163	0.100
PRB961	7	0.161	0.181	0.168	0.100
PRB961	12	0.165	0.188	0.176	0.100
PRB961	14	0.183	0.185	0.190	0.100
PRB961	19	0.180	0.187	0.164	0.100
PRB961	21	0.190	0.184	0.199	0.100
PRB961	27	12.4	13.0	12.5	8.80
PRB961	29	46.5	48.1	46.1	28.7
PRB966	0	0.158	0.171	0.174	0.100
PRB966	2	0.158	0.175	0.167	0.200
PRB966	20	0.166	0.163	0.172	0.200
PRB966	22	0.175	0.156	0.172	0.100
PRB966	30	0.177	0.175	0.172	0.200
PRB966	35	0.176	0.176	0.179	0.200
PRB966	37	0.227	0.246	0.248	0.300
PRB966	44	5.65	6.00	5.49	2.10
PRB966	48	2.04	2.04	1.90	2.10
PRB966	51	17.7	16.9	14.4	9.60

References

1 Barré-Sinoussi F, Chermann JC, Rey F, et al. Isolation of a T-lymphotrophic Retrovirus from a Patient at Risk for Acquired Immune Deficiency Syndrome (AIDS). Science 1983;220:868-871.

2 Popovic M, Sarngadharan MG, Read E, et al. Detection, Isolation and Continuous Production of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and Pre-AIDS. Science 1984;224:497-500.

3 Gallo RC, Salahuddin SZ, Popovic M, et al. Frequent Detection and Isolation of cytopathic Retroviruses (HTLV-III) from Patients with AIDS and Risk for AIDS. Science 1984;224:500-503.

4 Clavel F, Guétard D, Brun-Vézinet F, et al. Isolation of a New Human Retrovirus from West Africa Patients with AIDS. Science 1986;233:343-346.

5 Guertler LG, Hauser PH, Eberle J, et al. A New Subtype of Human Immunodeficiency Virus Type 1 (MVP-5180) from Cameroon. J Virol 1994;68(3):1581-1585.

6 Simon F, Mauclère P, Roques P, et al. Identification of a new human immunodeficiency virus type 1 distinct from group M and group O. Nature Medicine 1998;4(9):1032-1037.

7 Plantier JC, Leoz M, Dickerson JE, et al. A new human immunodeficiency virus derived from gorillas. Nature Medicine 2009;15(8):871-872.

8 Robertson DL, Anderson JP, Bradac JA, et al. HIV-1 nomenclature Proposal. Science 2000;288(5463):55-56.

9 Yamaguchi J, Vallari A, McArthur C, et al. Brief Report: Complete Genome Sequence of CG-0018a-01 Establishes HIV-1 Subtype L. J Acquir Immune Defic Syndr. 2020 Mar 1;83(3):319-322.

10 Taylor BS, Hammer SM. The challenge of HIV-1 subtype diversity. N Engl J Med 2008;358:1590-1602.

11 Guertler LG. Difficulties and strategies of HIV diagnosis. Lancet 1996;348:176-179.

12 Verdier M, Denis F, Leonard G, et al. Comparison of 10 Enzyme Immunoassays for Detection of Antibody to Human Immunodeficiency Virus Type 2 in West African Sera. J Clin Microbiol 1988;26:1000-1004.

13 Fiebig EW, Wright DJ, Rawal BD, et al. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. AIDS 2003;17(13):1871-1879.

14 Busch MP, Lee LL, Satten GA, et al. Time course of detection of viral and serologic markers preceding human immunodeficiency virus type 1 seroconversion: implications for screening of blood and tissue donors. Transfusion 1995;35:91-97.

15 Busch MP, Satten GA. Time course of viremia and antibody seroconversion following human immunodeficiency virus exposure. Am J Med 1997;102(5B):117-124.

16 Weber B, Fall EH, Berger A, et al. Reduction of Diagnostic Window by New Fourth-generation Human immunodeficiency Virus Screening Assays. Clin Microbiol 1998;36(8):2235-2239.

17 Guertler L, Mühlbacher A, Michl U, et al. Reduction of the diagnostic window with a new combined p24 antigen and human immunodeficiency virus antibody screening assay. Journal of Virological Methods 1998;75:27-38.

18 Occupational Safety and Health Standards: Bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.

19 Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

For further information, please refer to the appropriate user guide for the analytical unit concerned, the respective application sheets and the Method Sheets of all necessary components (if available in your country).

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

The following symbols and signs are used in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
	Global Trade Item Number
Rx Only	US only: Federal law restricts this device to sale by or on the order of a physician.

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