HIV Ag

cobas®

HIV Ag - human immunodeficiency virus type 1 (groups M and O) p24 antigen

REF	Σ	SYSTEM
		Elecsys 2010
		MODULAR ANALYTICS E170
11971611 122	100	**cobas e** 411
		cobas e 601
		cobas e 602

English

Intended use

Immunoassay for the in vitro qualitative determination of the p24 antigen of human immunodeficiency virus type 1 (HIV-1, groups M and O) in human serum and plasma and in cell culture supernates.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

References[1,2,3,4,5,6,7,8,9]

The human immunodeficiency virus (HIV) belongs to the group of retroviruses. So far, two types of human immunodeficiency virus have been described: HIV-1 and HIV-2. Within the known HI-viruses there are various subtypes showing geographical differences in their dissemination. For HIV-1 it is currently possible on the basis of genetic relationships to identify at least 9 different subtypes (A-I, also summarized as group M) in addition to the highly divergent O-group.

Following infection with HI-viruses, antibodies to proteins of the HI-virus generally appear in the serum after a variable period of time. Antibodies to HIV are indicative of an existing HIV infection.

Before the appearance of antibodies in the blood, free viruses may be present which can be detected by the p24 antigen test. The time elapsing before the p24 antigen can be detected is generally 3-5 weeks; about 30-50 % of persons infected with HIV show measurable antigenemia in the early phase of the infection. HIV p24 antigen can also be detectable in the late phase of HIV disease (AIDS) as a result of excessive viremia.

Due to the passive transfer of antibodies from mother to child, antibodies to HIV cannot be utilized in the detection of an HIV infection in the newborn. If HIV is passed on to a child from its HIV-infected mother, then the detection of HIV p24 antigen is a sign of an HIV infection in the newborn baby.

The HIV antigen test serves to identify an HIV infection

- in association with the anti-HIV antibody screening test in persons known to be at risk of contracting an HIV infection
- in newborn babies of mothers infected with HIV and
- to support monitoring during antiviral therapy.

The Elecsys HIV Ag assay employs monoclonal antibodies to determine the HIV-1 p24 antigen.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: HIV p24 antigen (from 50 µL of sample), a biotinylated monoclonal HIV p24-specific antibody, and a monoclonal HIV p24-specific antibody labeled with a ruthenium complex[a)] react to form a sandwich complex.
- 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/ProCell 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 reaction product of the sample with the signal of the cutoff value previously obtained by calibration.

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

Reagents - working solutions

The reagent rackpack (M, R1, R2) is labeled as HIVAG.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-HIV p24-Ab~biotin (gray cap), 1 bottle, 8 mL:
Biotinylated monoclonal anti-HIV p24 antibodies (mouse) > 0.5 mg/L; TRIS buffer 50 mmol/L, pH 7.4; preservative.

R2 Anti-HIV p24-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 8 mL:
Monoclonal anti-HIV p24 antibodies (mouse) labeled with ruthenium complex > 0.8 mg/L; TRIS buffer 50 mmol/L, pH 7.4; preservative.

HIVAG Cal1 Negative calibrator (white cap), 2 bottles of 1.0 mL each:
Human serum; preservative.

HIVAG Cal2 Positive calibrator (black cap), 2 bottles of 1.0 mL each:
HIV p24 antigen (E. coli, rDNA) approximately 240 pg/mL in acetate buffer, pH 4.5; preservative.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

All human material should be considered potentially infectious.

The negative calibrator has 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 applied were FDA-approved or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

However, as no 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 patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[10,11]

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

Reagent handling

The reagents in the kit are ready for use and are supplied in bottles compatible with the system.

Elecsys 2010 and **cobas e** 411 analyzers: The calibrators should only be left on the analyzers during calibration at 20-25 °C. After use, close the bottles as soon as possible and store upright at 2-8 °C.

Due to possible evaporation effects, not more than 5 calibration procedures per bottle set should be performed.

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Unless the entire volume is necessary for calibration on the analyzers, transfer aliquots of the ready-for-use calibrators into empty snap-cap bottles (CalSet Vials). Attach the supplied labels to these additional bottles. Store the aliquots at 2-8 °C for later use.

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

All information required for correct operation is read in from the respective reagent barcodes.

Please note: Both the vial labels, and the additional labels (if available) contain 2 different barcodes. The barcode between the yellow markers is for **cobas** 8000 systems only. If using a **cobas** 8000 system, please turn the vial cap 180° into the correct position so the barcode can be read by the system. Place the vial on the instrument as usual.

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Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:

Stability of the reagent rackpack	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	4 weeks
on MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602	4 weeks
on Elecsys 2010 and **cobas e** 411	4 weeks

Stability of the calibrators	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	4 weeks
on Elecsys 2010 and **cobas e** 411 at 20-25 °C	up to 5 hours
on MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 at 20-25 °C	use only once

Store calibrators **upright** in order to prevent the calibrator solution from adhering to the snap-cap.

Specimen collection and preparation

Only the specimens listed below were tested in a sufficient number and found acceptable.

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

Sodium heparin, K_3-EDTA and sodium citrate plasma.

Criterion: Correct assignment of positive and negative samples.

Stable for 10 days at 2-8 °C, 3 months at -20 °C. The samples may be frozen 5 times.

The sample types listed were tested with a selection of sample collection tubes 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 manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See “Reagents – working solutions” section for reagents.

- 2 x 4 bottle labels

Materials required (but not provided)

- REF 05162645190, PreciControl HIV, for 2 x 2 mL each of PreciControl HIV 1, 2, and 3 (do not use control 2 for the Elecsys HIV Ag assay)
- REF 12001101122, HIV Ag Confirmatory Test, 2 x 1 mL each of confirmatory reagent and control reagent
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for Elecsys 2010 and **cobas e** 411 analyzers:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- REF 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500316, 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 analyzer concerned. Refer to the appropriate operator’s manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Place the calibrators in the sample zone.

All the information necessary for calibrating the assay is automatically read into the analyzer.

After calibration has been performed, store the calibrators at 2-8 °C or discard (MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers).

Calibration

Traceability: This method has been standardized against HIV-1 p24 Antigen, 1st International Reference Reagent, NIBSC code: 90/636.

Calibration frequency: Calibration must be performed once per reagent lot using HIV Ag Cal1, HIV Ag Cal2 and fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).

Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required, e.g. quality control findings with PreciControl HIV outside the defined limits
- more frequently when this is required by pertinent regulations

Range for the electrochemiluminescence signals (counts) for the calibrators:

Negative calibrator (HIVAG Cal1): 600-1400,
positive calibrator (HIVAG Cal2): 18000-55000.

Quality control

For quality control, use PreciControl HIV (do not use PC HIV2 for the Elecsys HIV Ag assay).

The controls 1 and 3 (PC HIV) for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

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The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Note:
For technical reasons re-assigned target values valid only for a specific reagent and control lot combination, must be entered manually on all analyzers (except for the **cobas e** 602 analyzer). Therefore always refer to the value sheet included in the reagent kit or PreciControl kit to make sure that the correct target values are used.

When a new reagent or control lot is used, the analyzer will use the original values encoded in the control barcodes.

Calculation

The analyzer automatically calculates the cutoff based on the measurement of HIVAG Cal1 and HIVAG Cal2.

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

Interpretation of the results

Samples with a cutoff index < 0.90 are non-reactive in the Elecsys HIV Ag assay. These samples are considered negative for HIV Ag and do not need further testing.

Samples with a cutoff index in the range ≥ 0.90 to < 1.0 are considered borderline for HIV Ag in the Elecsys HIV Ag assay.

Samples with a cutoff index ≥ 1.0 are considered reactive in the Elecsys HIV Ag assay.

All initially reactive or borderline samples must be retested in duplicate with the Elecsys HIV Ag assay.

If these samples yield mean cutoff index values of < 0.90 upon redetermination, then they are deemed negative for HIV Ag.

Initially reactive or borderline samples with a cutoff index ≥ 0.90 in either of the redeterminations are considered repeatedly reactive.

Repeatedly reactive samples must be investigated using an independent neutralization test (Elecsys HIV Ag Confirmatory Test).

Samples confirmed by neutralization with human anti-HIV antibodies are regarded as positive for HIV Ag.

Diagnosis of existing HIV infection: In the event of an isolated positive HIV antigen test, the HIV infection should be confirmed in follow-up samples and by the anti-HIV screening test. A negative test result does not completely rule out the possibility of infection with the HI-virus.

Limitations - interference

The assay is unaffected by icterus (bilirubin < 428 µmol/L or < 25 mg/dL), hemolysis (Hb < 1.2 mmol/L or < 2.0 g/dL), lipemia (Intralipid < 1500 mg/dL) and biotin (< 409 nmol/L or < 100 ng/mL).

Criterion: Correct assignment of negative and positive samples.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 2400 IU/mL.

There is no high-dose hook effect at HIV Ag concentrations up to 200000 pg/mL.

In vitro tests were performed on 19 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Detection limit (cut-off sensitivity) of 0.5 IU/mL was established with HIV-1 p24 Antigen, 1st International Reference Reagent, NIBSC code: 90/636.

Expected values

In randomly selected blood donors (from a blood bank in Salzburg), the number of repeatedly reactive samples in the Elecsys HIV Ag assay was < 0.2 %.

Using the Elecsys HIV Ag assay, the presence of HIV Ag was demonstrated in 327 out of 329 cases of HIV-reactive samples.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, human sera and controls.

Elecsys 2010 and **cobas e** 411 analyzers						
	Repeatability[b]			**Intermediate precision**[c]		
Sample	Mean COI[d]	SD COI	CV %	Mean COI	SD COI	CV %
HS[e], negative	0.44	0.02	5.2	0.45	0.03	7.1
HS, weakly positive	2.26	0.06	2.7	1.88	0.07	3.7
HS, positive	73.5	1.90	2.6	29.9	1.23	4.1
PreciControl HIV Ag 1	0.42	0.03	6.6	0.52	0.03	5.9
PreciControl HIV Ag 2	53.2	1.07	2.0	46.9	2.33	5.0

b) Repeatability = within-run precision (n = 21)

c) Intermediate precision = between-run (n = 9)

d) COI = cutoff index

e) HS = human serum

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers						
	Repeatability[f]			**Intermediate precision**[g]		
Sample	Mean COI	SD COI	CV %	Mean COI	SD COI	CV %
HS, negative	0.19	0.01	7.6	0.17	0.02	11.7
HS, weakly positive	6.38	0.05	0.9	6.34	0.30	4.8
HS, positive	107	4.37	4.1	102	4.01	3.9
PreciControl HIV Ag 1	0.32	0.02	4.7	0.31	0.02	5.4
PreciControl HIV Ag 2	39.0	0.62	1.6	41.0	1.08	2.6

f) Repeatability = within-run precision (n = 21)

g) Intermediate precision = within-laboratory (modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 6 times daily for 10 days (n = 60))

Method comparison

A comparison of the Elecsys HIV Ag method with a commercial HIV Ag test using 513 samples from persons known to be infected with HIV showed the following result:

	Elecsys HIV Ag assay		HIV Ag comparison test	
Samples	reactive	non-reactive	reactive	non-reactive
HIV Ag positive	327	2	246	83
HIV Ag negative	0	184	0	184

Analytical specificity

No cross-reactions with HAV, HBV, HCV, HTLV, CMV, E. coli, Toxoplasma gondii, Rubella, and multiple myeloma were observed.

Measurements were performed on each of the pathogens listed above using ≥ 9 serum or plasma samples which were positive for antibodies to the above-mentioned pathogens or contained autoantibodies (e.g. AMA, MS, and other autoimmune diseases).

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Out of 16 EBV-positive and 20 HSV-positive samples, one in each group was found to give a false-positive reaction with the Elecsys HIV Ag assay.

Sensitivity (cell culture)

61 out of 61 cell culture supernatants of different HIV-1 subtypes and HIV-2 were correctly identified as positive.

Clinical sensitivity

Of 329 HIV Ag-positive samples from HIV-infected patients in various stages of the disease, 327 were found to be repeatedly reactive in the Elecsys HIV Ag assay.

Clinical specificity

The specificity of the Elecsys HIV Ag assay in the blood donor group was found to be as follows: initially reactive (IR) specificity 99.81 %; repeatedly reactive (RR) specificity 99.84 %.

Group	N	Reactive	Repeatedly reactive	Confirmed positive
Blood donors	2565	5	4	0
Hospitalized patients	150	2	2	0
Dialysis patients	50	1	1	0
Pregnant women	50	0	0	0

References

1 "Akte AIDS" Supplement of the MMW to the World AIDS Day 1 Dec. München Med Wschr 1997;139 Suppl 1:1-129.

2 Hammer S, Crumpacker C, D'Aquila R, et al. Use of Virologic Assays for Detection of Human Immunodeficiency Virus in Clinical Trials: Recommendation of the AIDS Clinical Trials Group Virology Committee. J Clin Microbiol 1993;31(10):2557-2564.

3 Chamberland ME, Castro KG, Haverkos HW, et al. Acquired Immunodeficiency Syndrome in the United States: An Analysis of Cases Outside High-Incidence Groups. Ann Intern Med 1984;101(5):617-623.

4 Arlievsky NZ, Pollack H, Rigaud M, et al. Shortened survival in infants vertically infected with human immunodeficiency virus with elevated p24 antigenemia. J Pediatr 1995;127(4):538-543.

5 Goudsmit J, Lange JM, Krone WJ, et al. Pathogenesis of HIV and its implications for the serodiagnosis and monitoring of antiviral therapy. J Virol Methods 1987;17(1/2):19-34.

6 Couroucé AM, Barin F, Maniez M, et al. Effectiveness of assays for antibodies to HIV and p24 antigen to detect very recent HIV infections in blood donors. AIDS 1992; 6(12):1548-1550.

7 Mulder JW, Lange JMA, de Wolf F, et al. Serum p24 antigen levels in untreated and zidovudine-treated HIV-1 infected subjects. J Med 1990;37:4-10.

8 Bowen PA, Lobel SA, Caruana RJ, et al. Transmission of Human Immunodeficiency Virus (HIV) by Transplantation: Clinical Aspects and Time Course Analysis of Viral Antigenemia and Antibody Production. Ann Int Med 1988;108:46-48.

9 Schüpbach J, Flepp M, Pontelli D, et al. Heat-mediated immune complex dissociation and enzyme-linked immunosorbent assay signal amplification render p24 antigen detection in plasma as sensitive as HIV-1 RNA detection by polymerase chain reaction. AIDS 1996;10(10):1085-1090.

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

11 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 operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

CE 0123

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