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HIV Ag Confirmatory Test

cobas®

HIV Ag Confirmatory Test

REF **12001101** 122 **2 x 20**

English

Intended use

Immunoassay for in vitro confirmation of the presence of HIV-p24 antigen in human serum and plasma samples repeatedly reactive or borderline when tested with the Elecsys HIV Ag assay.

Summary

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

The Elecsys HIV Ag Confirmatory Test is based on the principle of specific antibody neutralization. Polyclonal HIV Ag-specific antibodies bind to the immunodominant epitopes of the HIV-p24 antigen and thereby block the binding sites for the antibodies used in the Elecsys HIV Ag assay.

Test principle

The test principle is based on pretreatment of the samples with confirmatory reagent and control reagent followed by the assay procedure using the Elecsys HIV Ag assay. The positive control, PreciControl HIV 3, should be run in parallel as a performance check.

Sample pretreatment:

- Samples found to be repeatedly reactive or borderline in the Elecsys HIV Ag assay are treated in parallel with confirmatory reagent and control reagent and then incubated. The excess anti-HIV antibodies in the confirmatory reagent neutralize any HIV Ag in the sample. In the subsequent Elecsys HIV Ag assay this leads to a reduction in the cutoff index (COI) value (signal of sample/cutoff) in comparison to the value obtained for the sample with control reagent which was measured in parallel.

HIV Ag assay:

- 1st incubation: HIV-p24 antigen in the pretreated sample/control (50 µL) reacts with a biotinylated, monoclonal HIV-p24 Ag-specific antibody, and a HIV-p24 Ag-specific antibody labeled with a ruthenium complex[a)] 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. This is followed by manual verification of the validity of the test and interpretation of the findings.

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

Reagents - working solutions

HIV Ag Confirmatory Test 1
Confirmatory reagent (black cap), 2 bottles of 1.0 mL each:
Human serum, positive for anti-HIV antibodies; neutralisation capacity (NC) > 90 % for PreciControl HIV 3; preservative.

HIV Ag Confirmatory Test 2
Control reagent (white cap), 2 bottles of 1.0 mL each:
Human serum, anti-HIV negative; 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 control reagent (HIV Ag Confirmatory Test 2) 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.

The serum containing anti-HIV used in the confirmatory reagent (HIV Ag Confirmatory Test 1) was tested for hepatitis B and hepatitis C infections. The findings were negative. The serum containing anti-HIV was inactivated using β-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 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. Avoid contamination. Store at 2-8 °C after use.

Storage and stability

Store at 2-8 °C.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	4 weeks

Specimen collection and preparation

Samples that were repeatedly reactive in the Elecsys HIV Ag assay.

The conditions regarding stability and specimen collection described for the Elecsys HIV Ag assay also apply here.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 11971611122, HIV Ag reagent kit for 100 tests
  (the materials required for performing the Elecsys HIV Ag assay are listed in the Elecsys HIV Ag Method Sheet)
- Elecsys 2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Assay

Sample pretreatment:

- For positive and borderline samples having a cutoff index ≥ 0.95
  200 µL sample + 50 µL confirmatory reagent
  200 µL sample + 50 µL control reagent
  Mix well.

The positive control, PreciControl HIV 3, should always be run in parallel as a performance check:

- For the control
  200 µL PreciControl HIV 3 + 50 µL confirmatory reagent
  200 µL PreciControl HIV 3 + 50 µL control reagent
  Mix well.

Incubation of the reactants:

30-120 minutes at 15-25 °C or overnight at 2-8 °C.

Elecsys HIV Ag assay:

The pretreated samples are placed in the sample zone and registered by entering the sample identification data.

The Elecsys HIV Ag assay is performed in accordance with the instructions given in the Method Sheet of the test reagent kit.

Calibration

For calibration, calibration frequency, and calibration verification, see data given in the Method Sheet for the Elecsys HIV Ag assay.

Quality control

PreciControl HIV 3 should always be run in parallel with the samples needing confirmation. For PreciControl HIV 1 the cutoff index from the Elecsys HIV Ag assay is required for verification of the confirmatory test.

Verification is done by the user.

For the Elecsys HIV Ag assay the conditions given in the Method Sheet apply.

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Calculation

The analyzer calculates the cutoff automatically on the basis of measurements on the two Elecsys HIV Ag calibrators (HIV Ag Cal1, HIV Ag Cal2) contained in the kit.

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

The cutoff index is needed for evaluation of the confirmatory test.

Limitations - interference

For the Elecsys HIV Ag assay the data given in the Method Sheet of the test reagents on "Limitations - interference" apply.

In very rare cases, influencing factors can lead to a reactive result in the Elecsys HIV Ag assay which is then not confirmed in the Elecsys HIV Ag Confirmatory Test although the sample contains minor concentrations of HIV-1 p24 antigen.

In HIV antibody-negative samples of persons showing signs of an HIV infection, HIV-RNA tests and/or tests with follow-up samples must be performed if the Elecsys HIV Ag assay revealed negative or false-reactive results to confirm the previously obtained results.

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

Evaluation and interpretation of the results

Verification of the validity of the test:

Prior to evaluation, the validity of the test must be verified. Evaluation can be made when, in addition to the conditions applying to the Elecsys HIV Ag assay, the following criteria are fulfilled:

- The neutralization (N) of PreciControl HIV 3 in the confirmatory reaction must be > 90 % of that in the control reaction:

$$N = \frac{\text{COI control react. PC HIV3 - COI confirmat. react. PC HIV3}}{\text{COI control react. PC HIV3 - COI PC HIV1 (HIV Ag assay)*}} \times 100$$

If N is < 90 %, it is necessary to check the test conditions. Where appropriate, repeat the test with fresh reagent.

- For evaluation of the sample to be valid, the cutoff index for the sample with the control reagent must be ≥ 0.9.

Evaluation and interpretation of the results:

- Calculation of neutralization - N (%):

$$N = \frac{\text{COI control react. sample - COI confirmat. react. sample}}{\text{COI control react. sample - COI PC HIV1 (HIV Ag assay)*}} \times 100$$

In order to confirm a positive result of a sample, the neutralization of the sample in the confirmatory reaction must be > 50 % of that for the control reaction with the sample.

Interpretation:

N < 50 % = negative or falsely reactive

N > 50 % = positive

* Cutoff index of untreated PreciControl HIV 1 used for quality control in the Elecsys HIV Ag assay.

Specific performance data

Representative data for manual sample pretreatment followed by assay on the Elecsys 2010 analyzer are shown below. Results obtained in individual laboratories may differ.

Precision

Precision of the manual test steps was determined using 3 sera of differing HIV Ag concentrations (8-10 times per sample with both the control and the confirmatory reagents). After a 30-minute period of incubation at 20 °C the pretreated samples were determined on the Elecsys 2010 analyzer using Elecsys reagents, calibrators, and controls.

Results from original Elecsys HIV Ag assay - without sample pretreatment (repeatability, n = 8-10):

Sample	Mean COI	SD COI	CV %
Human serum, negative	0.46	0.04	9.4
Human serum, weakly positive	5.06	0.14	2.8
Human serum, positive	58.0	1.56	2.7
PreciControl HIV 3	141	1.31	0.9

Results after manual sample pretreatment:

	Control reaction		
Sample	Mean COI	SD COI	CV %
Human serum, negative	0.44	0.03	7.0
Human serum, weakly positive	4.13	0.12	2.9
Human serum, positive	31.5	1.22	3.9
PreciControl HIV 3	68.3	0.70	1.0

Results after manual sample pretreatment:

	Confirmatory reaction		
Sample	Mean COI	SD COI	CV %
Human serum, negative	0.42	0.04	8.8
Human serum, weakly positive	0.46	0.03	7.4
Human serum, positive	0.64	0.06	9.5
PreciControl HIV 3	0.43	0.02	4.6

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.

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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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