ms_07173032190V2.0

HTLV-I/II

cobas®

Antibody to human T-lymphotropic virus type I/II

REF	Σ	SYSTEM
07173032 190	200	MODULAR ANALYTICS E170 **cobas e** 411 **cobas e** 601 **cobas e** 602

English

Intended use

Immunoassay for the in vitro qualitative determination of antibodies to HTLV-I/II in human serum and plasma.

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

Regulatory approval

This assay has been CE marked according to Directive 98/79/EC. Test performance has been established and certified by a Notified Body according to the Common Technical Specifications (CTS) for diagnostic use and for screening of blood donations.

Summary

Human T-lymphotropic virus (HTLV) type I and II are two closely related retroviruses with 70 % nucleotide sequence homology.[1] HTLV-I comprises the different subtypes A-F. The geographic areas of the highest prevalence are Japan, Africa, the Caribbean islands and South America. Additional endemic regions include the Middle East and the Melanesian islands including Papua New Guinea.[2] HTLV-II comprises two main subtypes, A and B.[3] Both are present in intravenous drug users in North America, Europe, and Asia and have been found sporadically in Africa. HTLV-II A is present in certain American Indian tribes of North, Central, and South America, including the Navajo and Pueblo in New Mexico and the Kayapo, Krahô, and Kaxuyana in Brazil.[4]

HTLV is transmitted from mother to child, between intravenous drug users by needle sharing, by hetero - or homosexual intercourse and contaminated blood products.

With a frequency of 15-30 %, mother-to-child transmission has a similar frequency as that of an untreated HIV-1 infection, and occurs predominantly in the postnatal period through breast milk.

Transmission by blood products is strictly cell-associated; the virus is not transmitted by plasma or plasma-derived products.[5] Recipients of contaminated blood seroconvert with a 40-60 % probability and an estimated seroconversion time of 51 days.[6] The majority of HTLV-I infected individuals remain lifelong asymptomatic carriers. Only 2-3 % of the HTLV-I infected individuals develop adult T-cell leukemia (ATL) and 0.25-4 % develop HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP).[7] Although less than 10 % of HTLV-I carriers progress to ATL or HAM/TSP, the diseases are generally severe and progressively incapacitating. The disease type correlates with the route of infection; breastfeeding has been associated with ATL, and HAM/TSP with blood transfusion.[1] There have been some reports describing a correlation between HTLV-II infection and different diseases[8,9] nevertheless the evidence is not nearly as clear as that for HTLV-I.

The Elecsys HTLV-I/II assay is used for screening of blood donors to ensure the safety of blood products and for diagnosis of HTLV infection.

Test principle

Double antigen sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 30 µL of sample, biotinylated HTLV-specific recombinant antigens (HTLV-I gp21 and HTLV-II p24) and HTLV-specific recombinant antigens (HTLV-I gp21 and HTLV-II p24) 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'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

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

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 12 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 HTLV-specific recombinant antigens (E. coli)~biotin (gray cap), 1 bottle, 15 mL:
Biotinylated HTLV-specific recombinant antigens (E. coli) > 0.3 mg/L; MES[b)] buffer 50 mmol/L, pH 6,2; preservative.

R2 HTLV-specific recombinant antigens (E. coli)~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 15 mL:
HTLV-specific recombinant antigens labeled with ruthenium complex > 0.3 mg/L; MES buffer 50 mmol/L, pH 6.2; preservative.

b) MES = 2-morpholino-ethane sulfonic acid

HTLV Cal1 Negative calibrator (white cap), 2 bottles (lyophilized) for 1.0 mL each:
Human serum, non reactive for anti-HTLV antibodies.

HTLV Cal2 Positive calibrator (black cap), 2 bottles (lyophilized) for 1.0 mL each:
Human serum, reactive for anti-HTLV antibodies.

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.

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

2-methyl-2H-isothiazol-3-one hydrochloride

EUH 208 May produce an allergic reaction.

Product safety labeling primarily follows EU GHS guidance.

All human material should be considered potentially infectious.

Both calibrators (HTLV Cal1 and HTLV 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 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 (except for HTLV Cal1 and HTLV Cal2) and are supplied in bottles compatible with the system.

HTLV Cal1 and HTLV Cal2: Carefully dissolve the contents of one bottle 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 bottles.

cobas e 411 analyzer: The reconstituted 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 calibrator 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 reconstituted 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.

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 of the reagent rackpack	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	8 weeks
on the analyzers	4 weeks

Stability of the calibrators	
lyophilized	up to the stated expiration date
reconstituted at 2-8 °C	4 weeks
on **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 and found acceptable.

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

Li-heparin, Na-heparin, K_2-EDTA, K_3-EDTA, ACD, CPD, CP2D, CPDA and Na-citrate plasma as well as K_2-EDTA plasma tubes containing separating gel.

Sampling devices containing liquid anticoagulants have a dilution effect resulting in lower cutoff-index (COI) values for individual patient specimens. In order to minimize dilution effects it is essential that respective sampling devices are filled completely according to manufacturer's instructions.

Criterion: mean recovery of positive samples within ± 20 % of serum value. Absolute deviation of samples with COI values from 0.00-1.0 within ± 0.2 COI.

Stable for 14 days at 2-8 °C, 5 days at 20 °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 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 manufacturer.

Centrifuge samples containing precipitates and frozen samples 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.

The performance of the Elecsys HTLV-I/II assay has not been established with cadaveric samples or body fluids other than serum and plasma.

Materials provided

See "Reagents – working solutions" section for reagents.

- 2 x 2 bottle labels
- 4 empty labeled snap-cap bottles

Materials required (but not provided)

- REF 07108133190, PreciControl HTLV, for 2 x 1 mL each of PreciControl HTLV 0, 1 and 2
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- General laboratory equipment
- Distilled or deionized water

Accessories for **cobas e** 411 analyzer:

- 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 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- 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.

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: PreClean M solution is necessary.

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 reconstituted calibrators in the sample zone.

HTLV-I/II

Antibody to human T-lymphotropic virus type I/II

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: No internationally accepted standard for HTLV-I/II exists. This method has been standardized against a Roche standard. The units have been selected arbitrarily.

Calibration frequency: Calibration must be performed once per reagent lot using HTLV Cal1, HTLV 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 HTLV outside the defined limits
- more frequently when this is required by pertinent regulations

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

Negative calibrator (HTLV Cal1): 350-3000 (**cobas e** 411 analyzer), 350-2000 (MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers)

Positive calibrator (HTLV Cal2): 20000-100000 (all analyzers)

Quality control

For quality control, use PreciControl HTLV.

All controls should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

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: The controls are not barcode-labeled and therefore have to be run like external controls. All values and ranges have to be entered manually. Please refer to the section "QC" in the operator's manual or to the online help of the instrument software.

Non-barcode labeled controls: Only one target value and range for each control level can be entered in the analyzer. The reagent lot-specific target values have to be re-entered each time a specific reagent lot with different control target values and ranges is used. Two reagent lots with different control target values and ranges cannot be used in parallel in the same run.

The exact lot-specific target values and ranges are printed on the enclosed (or electronically available) value sheet in the reagent kit or PreciControl kit.

Please make sure that the correct values are used.

Calculation

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

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).

Interpretation of the results

Samples with a cutoff index < 1.00 are non-reactive in the Elecsys HTLV-I/II assay. These samples are considered negative for HTLV-I/II-specific antibodies and do not need further testing.

Samples with a cutoff index ≥ 1.00 are considered reactive in the Elecsys HTLV-I/II assay.

All initially reactive samples should be redetermined in duplicate with the Elecsys HTLV-I/II assay. If cutoff index values < 1.00 are found in both cases, the samples are considered negative for HTLV-specific antibodies. Initially reactive samples giving cutoff index values of ≥ 1.00 in either of the redeterminations are considered repeatedly reactive.

Repeatedly reactive samples must be confirmed according to recommended confirmatory algorithms. Confirmatory tests include Western Blot and HTLV PCR tests.

Limitations - interference

The assay is unaffected by icterus (bilirubin ≤ 1129 µmol/L or ≤ 66 mg/dL), hemolysis (Hb ≤ 0.3 mmol/L or ≤ 0.5 g/dL), lipemia (Intralipid ≤ 2000 mg/dL) and biotin (≤ 246 nmol/L or ≤ 60 ng/mL).

Criterion: Mean recovery of positive samples within ± 15 %. Absolute deviation of samples with COI values from 0.00-1.0 within ± 0.2 COI.

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 1200 IU/mL.

Studies have been performed to assess the high-dose hook effect. Out of 1149 positive samples no false negative result was found. Occurrence of high-dose hook effect cannot be completely excluded.

In vitro tests were performed on 17 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.

A negative test result does not completely rule out the possibility of an infection with HTLV-I/II. Serum or plasma samples from the very early (pre-seroconversion) phase or the late phase of HTLV-I/II infection can occasionally yield negative findings.

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, samples and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplication each for 21 days (n = 84). The following results were obtained:

cobas e 411 analyzer					
		Repeatability[c]		**Intermediate precision**[d]	
Sample	Mean COI	SD COI	CV %	SD COI	CV %
HS[e] 1	1.25	0.014	1.1	0.047	3.8
HS 2	0.836	0.010	1.2	0.031	3.8
HS 3	2.14	0.019	0.9	0.070	3.3
HS 4	1.43	0.014	1.0	0.052	3.6
HS 5	0.176	0.008	4.4	0.013	7.2
HS 6	0.151	0.004	2.9	0.008	5.0
HS 7	26.6	0.340	1.3	1.03	3.9
HS 8	5.34	0.092	1.7	0.203	3.8
PC[f] HTLV 0	0.192	0.008	4.4	0.013	6.5
PC HTLV 1	5.04	0.053	1.0	0.176	3.5
PC HTLV 2	2.34	0.027	1.2	0.083	3.6

c) Repeatability = within-run precision

d) Intermediate precision = between-run

e) HS = human serum

f) PC = PreciControl

HTLV-I/II

Antibody to human T-lymphotropic virus type I/II

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean COI	SD COI	CV %	SD COI	CV %
HS 1	1.29	0.014	1.1	0.021	1.6
HS 2	0.829	0.008	1.0	0.015	1.8
HS 3	2.28	0.023	1.0	0.037	1.6
HS 4	1.48	0.016	1.1	0.023	1.6
HS 5	0.076	0.001	1.2	0.002	2.3
HS 6	0.076	0.001	1.1	0.002	2.2
HS 7	29.8	0.279	0.9	0.488	1.6
HS 8	5.84	0.064	1.1	0.097	1.7
PC HTLV 0	0.083	0.001	1.4	0.002	2.6
PC HTLV 1	5.47	0.050	0.9	0.119	2.2
PC HTLV 2	2.48	0.020	0.8	0.055	2.2

Analytical specificity

222 samples containing potentially interfering substances were tested with the Elecsys HTLV-I/II assay comprising specimens containing antibodies:

- against HIV, EBV, HSV-1/2, Rubella, HAV, HBV, HCV, E. coli
- from autoimmune diseases (e.g. ANA) and elevated titers of rheumatoid factor

No false reactive results were found with the Elecsys HTLV-I/II assay resulting in a specificity of 100 %. Two samples were found repeatedly reactive with the Elecsys HTLV-I/II assay and were confirmed positive with HTLV immunoblot.

Clinical sensitivity

Of 1149 samples from HTLV-I/II infected patients of different geographical origin in different stages of the disease 1149 were found to be repeatedly reactive with the Elecsys HTLV-I/II assay. The sensitivity of the Elecsys HTLV-I/II assay in this study was 100 %.

Cohorts (by geographical origin)	N	Confirmed positive samples	Sensitivity %
Japan	420	420	100
South America	134	134	100
Carribean	97	97	100
USA	259	259	100
Europe/Middle East	236	236	100
Africa	3	3	100

Cohorts (summarized by virus type)	N	Confirmed positive samples	Sensitivity %
Total HTLV I	926	926	100
Total HTLV II	200	200	100
Total HTLV type unknown	23	23	100
Total	1149	1149	100

Clinical specificity

A total of 13974 samples (diagnostic routine, pregnant women and blood donors) from 6 centers in Europe and Japan were tested with the Elecsys HTLV-I/II assay. The resulting specificity in the study was 99.95 % in blood donors (n = 11575) and 99.83 % in diagnostic routine including pregnant women (n = 2399). The 95 % lower confidence limit was 99.89 % in blood donors and 99.56 % in diagnostic routine including pregnant women.

Cohort	N	Confirmed positive samples	Indeterminate samples	Specificity[g] %
Blood donor serum	9551	1	2	99.94 (99.86-99.98)
Blood donor EDTA plasma	2024	0	1	100 (99.82-100)
Diagnostic routine (including pregnant women)	2399	59	3	99.83 (99.56-99.95)

g) 95 % confidence interval, two sided

References

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8 Murphy EL, Wang B, Sacher RA, et al. Respiratory and Urinary Tract Infections, Arthritis, and Asthma Associated with HTLV-I and HTLV-II Infection. Emerg Infect Dis 2004;10:109-116.

9 Zehender G, Colasante C, Santambrogio S, et al. Increased Risk of Developing Peripheral Neuropathy in Patients Coinfected With HIV-1 and HTLV-2. JAIDS 2002;31:440-447.

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

ms_07173032190V2.0

HTLV-I/II

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CE 0123

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