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 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 chemiluminescence 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.

Reagents – working solutions

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

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

- R1 HTLV-specific recombinant antigens (E. coli) biotin (gray cap), 1 bottle, 8 mL:
  - Biotinylated HTLV-specific recombinant antigens (E. coli) > 0.3 mg/mL: MES buffer 50 mmol/L, pH 6.2; preservative

- R2 HTLV-specific recombinant antigens (E. coli) Ru(bpy)3 (black cap), 1 bottle, 8 mL:
  - HTLV-specific recombinant antigens labeled with ruthenium complex > 0.3 mg/L; MES buffer 50 mmol/L, pH 6.2; 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.

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.
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.

**cobs 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, cobs e 601 and cobs 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.

**Perfor 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 cobs 8000 systems only. If using a cobs 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 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 cobs e 411 at 20-25 °C: up to 5 hours
- on MODULAR ANALYTICS E170, cobs e 601 and cobs 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 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.

Criterions: 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 cobs 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 AssyCup, 60 x 60 reaction vessels
- Ref 11706799001, Elecsys 2010 AssyTip, 30 x 120 pipette tips

**Accessories for MODULAR ANALYTICS E170, cobs e 601 and cobs 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, PreciClean M, 5 x 600 mL detection cleaning solution
- Ref 12102137001, AssyTipAssayCup 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, cobs e 601 and cobs e 602 analyzers: PreClean M solution is necessary.
Antibody to human T-lymphotropic virus type I/II

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.

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 lot 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 specific target values and ranges.

If necessary, repeat the measurement of the samples concerned.

**Interference**

- 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.
- 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:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean COI</th>
<th>SD COI</th>
<th>CV %</th>
<th>SD COI</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>HS&lt;sup&gt;a&lt;/sup&gt; 1</td>
<td>1.25</td>
<td>0.014</td>
<td>1.1</td>
<td>0.047</td>
<td>3.8</td>
</tr>
<tr>
<td>HS 2</td>
<td>0.836</td>
<td>0.010</td>
<td>1.2</td>
<td>0.031</td>
<td>3.8</td>
</tr>
<tr>
<td>HS 3</td>
<td>2.14</td>
<td>0.019</td>
<td>0.9</td>
<td>0.070</td>
<td>3.3</td>
</tr>
<tr>
<td>HS 4</td>
<td>1.43</td>
<td>0.014</td>
<td>1.0</td>
<td>0.052</td>
<td>3.6</td>
</tr>
<tr>
<td>HS 5</td>
<td>0.176</td>
<td>0.008</td>
<td>4.4</td>
<td>0.013</td>
<td>7.2</td>
</tr>
<tr>
<td>HS 6</td>
<td>0.151</td>
<td>0.004</td>
<td>2.9</td>
<td>0.008</td>
<td>5.0</td>
</tr>
<tr>
<td>HS 7</td>
<td>26.6</td>
<td>0.340</td>
<td>1.3</td>
<td>1.03</td>
<td>3.9</td>
</tr>
<tr>
<td>HS 8</td>
<td>5.34</td>
<td>0.092</td>
<td>1.7</td>
<td>0.203</td>
<td>3.8</td>
</tr>
<tr>
<td>PO&lt;sup&gt;b&lt;/sup&gt; HTLV 0</td>
<td>0.192</td>
<td>0.008</td>
<td>4.4</td>
<td>0.013</td>
<td>6.5</td>
</tr>
<tr>
<td>PC HTLV 1</td>
<td>5.04</td>
<td>0.053</td>
<td>1.0</td>
<td>0.176</td>
<td>3.5</td>
</tr>
<tr>
<td>PC HTLV 2</td>
<td>2.34</td>
<td>0.027</td>
<td>1.2</td>
<td>0.083</td>
<td>3.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> Repeatability = within-run precision
<sup>b</sup> Intermediate precision = between-run
<sup>c</sup> HS = human serum
<sup>d</sup> PC = PreciControl
<sup>e</sup> 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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Repeatability</th>
<th>Intermediate precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean COI SD COI CV %</td>
<td>SD COI CV %</td>
</tr>
<tr>
<td>HS 1</td>
<td>1.29 0.014 1.1</td>
<td>0.021 1.6</td>
</tr>
<tr>
<td>HS 2</td>
<td>0.829 0.008 1.0</td>
<td>0.015 1.8</td>
</tr>
<tr>
<td>HS 3</td>
<td>2.26 0.023 1.0</td>
<td>0.037 1.6</td>
</tr>
<tr>
<td>HS 4</td>
<td>1.48 0.016 1.1</td>
<td>0.023 1.6</td>
</tr>
<tr>
<td>HS 5</td>
<td>0.076 0.001 1.2</td>
<td>0.002 2.3</td>
</tr>
<tr>
<td>HS 6</td>
<td>0.076 0.001 1.1</td>
<td>0.002 2.2</td>
</tr>
<tr>
<td>HS 7</td>
<td>29.8 0.279 0.9</td>
<td>0.488 1.6</td>
</tr>
<tr>
<td>HS 8</td>
<td>5.84 0.064 1.1</td>
<td>0.097 1.7</td>
</tr>
<tr>
<td>PC HTLV 0</td>
<td>0.083 0.001 1.4</td>
<td>0.002 2.6</td>
</tr>
<tr>
<td>PC HTLV 1</td>
<td>5.47 0.050 0.9</td>
<td>0.119 2.2</td>
</tr>
<tr>
<td>PC HTLV 2</td>
<td>2.48 0.020 0.8</td>
<td>0.055 2.2</td>
</tr>
</tbody>
</table>

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 titters 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%.

Cohort | N | Confirmed positive samples | Indeterminate samples | Specificity % |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood donor serum</td>
<td>9551</td>
<td>1</td>
<td>2</td>
<td>99.94 (99.86-99.98)</td>
</tr>
<tr>
<td>Blood donor EDTA plasma</td>
<td>2024</td>
<td>0</td>
<td>1</td>
<td>100 (99.82-100)</td>
</tr>
<tr>
<td>Diagnostic routine (including pregnant women)</td>
<td>2399</td>
<td>59</td>
<td>3</td>
<td>99.83 (99.56-99.95)</td>
</tr>
</tbody>
</table>

References

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.

Contents of kit
- Analyzers/Instruments on which reagents can be used
- Reagent
- Calibrator
- Volume after reconstitution or mixing

Global Trade Item Number

Roche Diagnostics GmbH, Sandwicher Strasse, 116, D-68330 Mannheim
www.roche.com