DRG HIV 1/2 ELISA (EIA-3491)

Revised 3 Aug. 2005 **Not for Sale in the USA**

The retroviruses HIV-1 and HIV-2 are known to be causative agents of the Acquired Immunodeficiency Syndrome (AIDS). Both retroviruses are transmitted mainly through sexual intercourse or through contact with blood and blood products as well as diaplacentally from pregnant women to their babies. More than 90 % of infected individuals develop antibodies to different proteins of HIV within a period of 3 to 6 weeks after infection. A short viraemic phase precedes seroconversion, in which p24 antigen is detectable by highly sensitive assays. From that point of time genomically integrated viral DNA can also be detected in mononuclear blood cells by polymerase chain reaction. However, the method of choice for screening of HIV infections is the detection of antibodies to HIV by enzyme immunoassay.

Test principle

The DRG anti-HIV-1/2 ELISA is a second generation solid-phase sandwich ELISA. Peptides corresponding to immunodominant regions of the transmembrane proteins gp41of HIV-1and gp36 of HIV-2 have been synthesized and the capsid protein p24 of HIV-1 was genetically engineered and expressed in *E. coli*. Together with a mixture of the synthetic peptides, the purified protein was used for adsorption to the inner surface of the microwell strips. Serum samples are diluted in the wells and incubated. If a specimen contains specific antibodies to HIV they will bind to the solid phase adsorbed peptides and the protein. Unbound serum components are removed by washing the wells and a mixture of horse radish peroxidase (HRP) labelled anti-human IgG and IgM antibodies is added to the wells. If complexes with anti-HIV antibodies of IgG or IgM isotypes are present in the wells the labelled anti-human antibodies will bind. After a further washing of the wells substrate solution (hydrogen peroxide and tetramethyl benzidine) is added. In all wells in which labelled antibodies were bound substrate reaction is mediated by HRP and the colourless substrate solution turns blue. After termination of the substrate reaction by addition of sulphuric acid to the wells the colour switches to yellow. The absorbance of the yellow product is determined by dual wavelength measurement at 450 nm and 620 nm or 690 nm in a microtitration plate reader.

Pept rAg	**+ sample (serum, plasma)**	**+ conjugate (anti-IgG/IgM-HRP)**	**+ substrate (TMB/H_2O_2)**	**+ stop (H_2SO_4)**	**→ 450 nm/620 nm yellow product**

Package size

One 96 well test kit to determine a maximum of 91 samples.

Stability

If stored correctly at 2-8°C reagents will be stable up to the expiry date stated on the labels.
The complete test kit will be stable for 18 months if reagents and strips remain closed and if the kit is stored at 2-8°C. Once opened the kit reagents should be stored at 2-8°C and used within 4 weeks.

Test kit components

1 **Microtitration strips with strip holder and plate sealers**
12 x 8 well strips, coated with synthetic peptides from immunodominant region of the trans-membrane protein gp41 of HIV-1 and of gp36 of HIV-2 and an authentic p24 recombinant antigen (*E. coli*) from HIV-1

2 **2 x 50 ml washing solution 10fold** (bottle with white cap)
Concentrate for 1000 ml, pH 7.3 ± 0.1

TRIS buffer	0.1	mol/l
Sodium chloride	3.0	mol/l

	Tween 20	1.0	% (v/v)

3 **30 ml dilution buffer** (bottle with black cap)
ready for use, pH 7.3 ± 0.1

TRIS buffer	0.01	mol/l
Sodium chloride	0.3	mol/l
Tween 20	0.1	% (v/v)
phenol red	0.001	% (w/v)
Animal serum (horse, calf)		

4 **0.75 ml negative control** (tube with green cap)
ready for use
inactivated human serum negative for anti-HIV antibodies, negative for HBs antigen and anti-HCV antibodies

5 **0.50 ml positive control** (tube with red cap)
ready for use, potentially infectious
inactivated human serum, positive for anti-HIV antibodies,
negative for HBs antigen and anti-HCV antibodies

6 **0.25 ml anti-human-IgG/IgM-HRP conjugate 101fold** (tube with brown cap)
concentrated solution, pH 7.3 ± 0.1
a mixture of anti-human IgG antibodies (sheep) and
anti-human IgM antibodies (sheep), labelled with HRP

7 **2.00 ml TMB stock solution 11fold** (glass tube with blue cap)
concentrated solution in DMSO/acetone
(aggressive to skin)

8 **15.00 ml substrate buffer (bottle with blue cap)**
ready for use, pH 4.0 ± 0.1

citrate buffer	0.2 mol/l
hydrogen peroxide	3 mmol/l

9 **15 ml stopping solution (bottle with yellow cap)**
ready for use

sulphuric acid	1 mol/l

(aggressive to skin)

Reagents contain the following preservatives:	0.01	% (w/v)	Gentamicin in 4 and 5
	0.01	% (w/v)	Thimerosal in 2 and 3
	0.1	% (w/v)	Sodium azide in 4 and 5
	1.0	% (v/v)	Kathon in 6
	0.01	% (v/v)	Kathon in 8

Reagent preparation

Preliminary comments
- The test kit is for *in vitro* use only.
- Do not use the test kit or opened reagents beyond the expiration date.
- Do not mix reagents from different lots.
- Do not use reagents produced by different manufacturers to complete a test kit.
- All reagents and the strips have to reach room temperature before use.
- Reagents not in use should always be stored at 2…8ºC.

Working washing solution
Dilute washing solution 10fold (2) 1 + 9 with fresh distilled water, e.g. 10 ml (2) + 90 ml dist. water. The working washing solution is stable for 1 week at 2-8ºC.

Conjugate working solution
Dilute the anti-human IgG/IgM-HRP conjugate 101fold (6) 1 + 100 with dilution buffer (3) e.g. 10 µl (6) + 1 ml (3) (sufficient for 1 strip). Prepare the conjugate working solution at least 15 min prior to use by mixing thoroughly. The conjugate working solution is stable for 1 day at room temperature.

Substrate working solution
Dilute the TMB stock solution (7) 1 + 10 with substrate buffer (8) e.g. 100 µl (7) + 1 ml (8) (sufficient for 1 strip). Prepare the substrate working solution 15 min prior to use by mixing thoroughly.
The substrate working solution is stable for 1 day at room temperature.
Caution: Contact with skin and eyes or metallic objects should be avoided.

Microtitration strips
The strips are vacuum sealed and stored in an outer plastic bag with a desiccant. Before opening, the strips must be at room temperature. Unused strips should be returned to the outer bag with the desiccant and resealed carefully.

Specimens

Serum or plasma
For optimum results non-hemolytic and non-lipemic samples should be tested immediately after collection. In case immediate testing is not possible samples may be stored for a maximum of 5 days at 2-8ºC, for longer periods at -20ºC.
Do not use heat-inactivated or detergent-treated samples.

Materials required but not provided

- adjustable micropipettes 0.010 to 1.000 ml
- multichannel pipette 0.100 ml
- pipette tips
- diluter dispenser (if available)
- incubator (37°C ± 1°C)
- automatic multichannel photometer (microtitration plate reader) with 450 and 620 or 690 nm filter
- washer (automatic plate washer or 8-channel manual microwell washer) with vacuum pump and waste bin
- basins as reservoir for liquid reagents for pipetting with a multichannel pipette
- flasks and cylinders for preparing the working solutions
- refrigerator for storage of test kits and unused reagents

Test procedure

Preliminary comments

- Follow the procedure exactly as described.
- Pay particular attention to the washing procedure.
- Bring all kit reagents to room temperature before use.
- Before commencing the assay all specimens and controls should be carefully recorded on the form supplied with the kit.
- Select the required number of microtitration strips out of the strip holder.
- Pipette tips have to be changed for each well to avoid cross contamination.
- Once the test has been started, all subsequent steps should be completed without interruption. This is of special importance for dispensing serum samples to avoid differing reaction times.

Precautions

- Handle all serum samples and control sera of the test kit as though capable of transmitting infection.
- Before disposing of all biological materials of the test kit including the tested sera and contaminated objects, handle them in a way to guarantee inactivation of HIV and hepatitis viruses (e.g. autoclaving for 60 min at 121°C, disinfection for 60 min with sodium hypochlorite at a 1 % final concentration, or for 60 min with peracetic acid in a 5 % final concentration.
- Wear working clothes (coat, rubber gloves)
- After the test has been completed, disinfect the working place for 60 min with 1 % sodium hypochlorite solution or with a 5 % peracetic acid.

Step 1

1. Wash the microtitration strips once immediately before use by adding
   400 µl **Working wash solution** per well. Incubate about 30 sec (soak time) and aspirate.
2. Add
   100 µl **Dilution buffer** into all wells
3. Dispense
   20 µl **Negative control** to wells A1, B1 and C1
   20 µl **Positive control** to wells D1 and E1
   20 µl patient samples into remaining wells.
   When pipettes are used aspirate the controls and all samples at least two times to homogenize the mixture.
4. Cover the strips with a plate sealer and incubate
   30 min at 37°C.
5. Prepare **Conjugate working solution.**
6. Aspirate the contents of the wells into 5 % sodium hypochlorite solution and add to each well
   400 µl **Working washing solution.**
   After about 30 sec soak time aspirate again and repeat this washing step 4 times. Prime automatic washers with working washing solution and wash strips 5 times. Ensure the washer fills all wells completely and aspirates efficiently after each 30 sec (remaining liquid: < 10 µl). Prior to the next working step remove remaining liquid completely by tapping the plate upside down on tissue paper.

Step 2

1. Dispense

100 µl **Conjugate working solution into all wells**
2. Cover the strips with a plate sealer and incubate
15 min at 37°C.
3. Prepare **Substrate working solution.**
4. Wash strips 5 times as described in step 1 no. 6.

Step 3

1. Dispense
100 µl **Substrate working solution** into all wells.
2. Incubate
15 min at room temperature (20…26°C).
3. Dispense
100 µl **Stopping solution into all wells.**
Measure absorbances within 30 min against air at
450 nm using a reference wavelength of 620 or 690 nm.

Calculation

Calculate the mean absorbance of the negative controls (MNC) and the positive controls (MPC) at 450/620 nm wavelength:

MNC = [A450 nm (A1) + A450 nm (B1) + A450 nm (C1)] : 3
MPC = [A450nm (D1) + A450 nm (E1)] : 2

Validation of the test

The test results are valid provided that the following criteria are met:

1. **MNC ≤ 0.15**
2. **MPC ≥ 1.50**

Determination of the cut-off and grey area

The cut-off value is determined as

MNC + 0.30

The gray area covers the absorbance from 90 % of the cut-off value to the cut off itself and is determined as

Cut-off value - (0.1 x cut-off value)

Evaluation of the samples

1. If the absorbance of a sample is lower than the lower borderline of the grey area the sample should be considered as non-reactive and the person the sample has been taken from as non-infected by HIV.

2. If the absorbance of a sample is higher than the cut-off value or is within the grey area, the sample has to be tested again in order to exclude procedural errors. If the sample is repeatedly reactive, a confirmatory test has to be carried out.
3. If the confirmatory test is positive, the person the sample has been collected from should be considered as being infected by HIV.
4. If the confirmatory test is negative, a new blood sample has to be taken after 14 days. This sample has to be tested in parallel to the first sample according to the procedure described above.

Limitations of the procedure

Cross contamination of reagents and samples can produce false results. Incorrect dilutions of reagents and samples, variation of incubation times and temperatures and incorrect washing of the wells as well as strong lipemic and hemolytic or microbial contaminated samples can cause false results.

Note: Negative test results in the anti-HIV-1/2 ELISA do not exclude HIV infection with absolute certainty.