++++ Change of ¼ ++++ Please read marked text carefully! ++++

HIV Ag/Ab

ELISA for the Qualitative Detection of Antibodies to Human Immunodeficiency Virus Types 1 and 2 (HIV-1, HIV-2), HIV-1 Group O and p24 Antigen in Human Serum and Plasma

Package Size

[REF] 51215 96 Tests Complete Test Kit

[IVD]

Intended Use

The HUMAN HIV Ag/Ab ELISA test has been developed to detect antibodies to HIV-1 (including group O) and HIV-2 and the HIV-1 p24 antigen in serum and plasma. The assay is intended for professional use for screening potentially infectious samples to prevent its use as donor materials.

Principle Direct antibody / antigen detection

The HUMAN HIV Ag/Ab ELISA is a double antigen and double antibody sandwich ELISA of the 4th generation. Recombinant antigens (HIV-rAg) specific for HIV-1 (gp160, p31, gp41 group O) and HIV-2 (gp36) as well as monoclonal antibodies against the HIV-1 p24 antigen (p24-mAb) are coated on the microtiter wells, whereas a mixture of biotinylated rAg HIV-1 (gp41, p31, gp41 group O), HIV-2 (gp36) and HIV-1 p24 mAb are used for Conjugate-1. During incubation HIV-specific antibodies (HIV-Ab: anti-HIV-1/2 IgG, IgM, IgA) and p24 antigen (HIV-Ag) present in patient specimen or the positive control, bind to both the immobilised HIV-rAg/p24-mAb and the Conjugate-1 forming double antigen-antibody complexes. These complexes are labelled by addition of Conjugate-2 (HRP labelled rAg HIV-1 gp41, HIV-2 gp36 and streptavidin). After the washing step for removal of unbound components, TMB/Substrate is added. A blue colour develops changing to yellow after stopping the reaction. The intensity of the colours is directly proportional to the HIV-Ab/HIV-Ag concentration in the specimen.

The absorbance of controls and specimen is determined by using ELISA microplate readers or automated ELISA systems at 450 nm. Results for patient samples are obtained by comparison with the cut-off value based on the negative control. HUMAN ELISA are compatible with both manual and automated applications (see notes).

Clinical value

Detection of antibodies to HIV proteins or simultaneous detection of antibodies and p24 core protein is the most prevalent method of HIV laboratory diagnostics. HIV antigens and antibodies appear and are detectable at different stages of the seroconversion and of the infection. Use of a highly sensitive kit for simultaneous detection of antibodies and p24 antigen allows reducing a phase of serological window on the average to 4-6 days due to the detection of the earliest marker of HIV infection p24 antigen. The diagnostic significance of p24 antigen is important also at last stage of an infection, when functional ability of immune system is low.

Reagents and Contents

[MIC]	12	Microtiter Strips (in strip holder) breakable 8-well strips coated with HIV-rAg (E. coli) and p24-mAb	
[NC]	2.5 ml	anti-HIV 1/2, p24 Negative Control (green cap) ready for use, human, dyed green Thimerosal	0.01%
[PC-1]	2.5 ml	p24 Positive Control (white cap) ready for use, human, dyed crimson-red ProClin 300	0.1%
[PC-2]	2.5 ml	anti-HIV 1/2 Positive Control (red cap) ready for use, human, dyed orange ProClin 300 Phenol	0.1% 0.01%
[CON-1]11x]	1.2 ml	anti-HIV 1/2, p24 Conjugate (yellow cap) Concentrate, HIV-rAg and p24-mAb, biotinylated Thimerosal Gentamicin sulphate	0.01% 0.01%
[CON-2]11x]	1.4 ml	HRP Conjugate (blue cap) Concentrate, HRP labelled HIV-rAg and streptavidin Phenol	0.02%
[DIL-C1]	12 ml	Conjugate-1 Diluent (transparent cap) NaCl, dyed orange Thimerosal Gentamicin sulphate Phenol	29.25 g/l 0.01% 0.01% 0.02%
[DIL-C2]	14 ml	Conjugate-2 Diluent (transparent cap) NaCl, dyed blue Thimerosal	34.0 g/l 0.01%
[WS]25x]	50 ml	Wash Solution (transparent cap) 25x concentrate, slightly opalescent Phosphate buffered saline Tween-20	2.5 %
[SA]11x]	2.5 ml	Substrate Reagent A (brown cap) Concentrate, 3,3', 5,5'-tetramethylbenzidin (TMB)	4.0 g/l
[SB]	25 ml	Substrate Reagent B (brown cap) Citrate-phosphate buffer Hydrogen peroxide ProClin 300	0.0076 % 0.04%
[STOP]	25 ml	Stop Solution (transparent cap) Sulphuric acid, ready for use	0.2 mol/l
	2	Adhesive protective film	
	1	Zip-lock plastic bag	

Additional materials recommended but not supplied with the kit

- Micropipettes, ELISA washer, incubator for 37°C ± 1.0°C, microplate reader equipped with 450 nm or with 450/620–680 nm filters, deionised water

Readers and Automated Analysers

Validated settings for the following HUMAN ELISA instruments are preinstalled or can be obtained from your local distributor. Application sheets for HUMAN instruments with analyser/assay-specific handling and performance information are accessible via: www.human.de/aps-elisa

HUMAN ELISA Instrument	Instrument type	REF
HumaReader Single plus	microplate reader	18000
HumaReader HS	microplate reader	16670
Elisys Uno	automated analyser	17350
Elisys Duo	automated analyser	17200
Elisys Quattro	automated analyser	16300

For automated analysers other than those provided by HUMAN follow the Pipetting scheme section and ensure all requirements described in the Procedural notes section have been satisfied. All protocols for automated analysers must be fully validated prior to use.

Safety Notes

All patient specimens and controls should be handled as potentially infectious. [PC-1] and [PC-2] have been inactivated by heating. [PC-1], [PC-2] and [NC] have been checked on donor level for the following parameters and were found to be non-reactive. [PC-1]: anti-HCV, HIV p24 Ag and HBsAg; [PC-2]: anti-HCV, anti-HIV-1/2 and HBsAg; [NC]: anti-HCV, anti-HIV-1/2, HIV p24 Ag and HBsAg. Wear protective clothing and disposable gloves.

All materials contaminated with patient specimens or controls, including [WASH] and other liquid and solid waste should be inactivated by validated procedures (autoclaving or chemical treatment) and dispose in accordance with applicable local regulations.

Storage / Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C, even after opening (see also "Note").

[MIC]

- Sealed in an aluminium bag.
- Allow to reach 18…24°C before opening!
- Return unused [MIC] into the aluminium bag and reseal the foil-lined package in zip-lock plastic bag. Do not remove desiccant. Store at 2...8°C.
- Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (18...24°C) before use. Reagents not in use should always be stored at 2...8°C.

Working wash solution [WASH]

- If crystals in [WS]25x] dissolve it by heating at 35...39°C (approx. 20 min.).
- Dilute [WS]25x] 1 + 24 with fresh deionised water, e.g. 40 ml [WS]25x] + 960 ml = 1000 ml.
- Stability: 14 days at 18...24°C or 28 days at 2…8°C.

Working conjugate-1 solution [WCON-1]

- In case of pellet of [DIL-C1] dissolve by mixing gently.
- Dilute thoroughly mixed [CON-1]11x] 1+10 with [DIL-C1]: e.g. 600 µl [CON-1]11x] + 6.0 ml [DIL-C1]. Mix thoroughly until diluted, avoid foaming, but do not apply intensive mixing. Stability: 12 hours at 18...24°C, in the dark.

Working conjugate-2 solution [WCON-2]

- Dilute thoroughly mixed [CON-2]11x] 1+10 with [DIL-C2]: e.g. dilute 700 µl [CON-2]11x] with 7.0 ml [DIL-C2]. Mix thoroughly until diluted, avoid foaming, but do not apply intensive mixing.
- Stability: 12 hours at 18...24°C, in the dark.

Substrate working solution [SUB]

- Dilute [SA]11x] 1+10 with [SB]: e.g. 1.2 ml [SA]11x] + 12 ml [SB].
- Use clean container only, rinsed with deionised water prior to use.
- Handle [SUB] carefully and avoid contamination! Do not use, if it appears blue!
- Stability: 10 hours at 18...24°C, when stored at a dark place.

Specimen

Use undiluted EDTA/heparin/citrate serum and plasma specimens. Do not use specimens that are heat-inactivated or pooled or highly lipaemic, or haemolysed or contain fibrin particles or sodium azide.

Note! Specimens with a hyperproteinaemia and hyperbilirubinaemia have not been evaluated.

For patients taking biotin dietary supplements or receiving a high dose biotin therapy (>5mg/day), specimens should be collected at least 8 hours after the last biotin administration.

Specimens may be stored for 48 hours at 2...8°C or longer at -20°C. Freeze and thaw no more than three times. Thaw quickly in a water bath at 40°C, but avoid temperatures above 40°C, due to instability of HIV Ag. Thawed specimens must be homogenised. Red cells or particulate matter should be eliminated by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: The temperature in the laboratory should be 18...24°C.

P2: Do not mix reagents from different lot numbers within a run. Do not mix caps of vials (risk of contamination). Do not let the wells dry once the assay has been started. Do not use reagents after their expiration date.

P3: Do not use reagents that could be contaminated or look or smell different than usual.

P4: Record specimens and controls carefully on the spread sheet supplied with the kit.

P5: [MIC] - select the required number of microwells.

P6: Using in a run less than 3 microtiter strips the number of controls can be reduced to 2 x [NC], 1 x [PC-1], 1 x [PC-2]. Pipette controls and specimen on the bottom in the microwells.

P7: Never use the same container for conjugate and solutions.

P8: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 15 minutes. If more than 1 plate is used, repeat the controls for each plate.

P9: Avoid/remove air bubbles prior to incubations and reading of absorbance.

P10: [SUB] – incubate in the dark. [SUB] initiates a kinetic reaction, which is terminated by [STOP].

P11: Do not use reagents without label or with damaged label / package

P12: Always firmly close vials with the proper caps after use

P13: Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc.

P14: Always store Stock solutions at 2...8°C when not in use

P15: Do not run the test in the presence of reactive vapours (acid, alkaline, aldehyde) or dust

Wash Procedure

The wash procedure is critical as insufficient washing will result in poor precision or falsely high absorbances. Use of an automatic microplate washer is strongly recommended.

W1: Aspirate off the contents into 5% sodium hypochlorite solution and add [WASH] to each well, aspirate off after at least 40 sec. soak time and repeat washing 3 times (Step 2).

W2: Fill and prime automatic washers with [WASH]. Subsequently wash strips 4 times (Step 2). Ensure the washer fills all wells completely and aspirates off efficiently after a soak time of at least 40 sec. (remaining liquid: < 10 µl).

W3: After washing, remove remaining liquid (use double aspiration in the final step where possible); avoid to tap out the plate.

W4: Do not allow the microwells to dry between the end of the washing operation and the subsequent [SUB] distribution.

Pipetting Scheme

Follow the procedure exactly as described. Pay particular attention to the washing procedure!				
The temperature in the laboratory should be 18...24°C. Before use keep reagents and specimens at least for 30 min. at room temperature				
Step 1	*Well [µl]*			
	A1-C1 [NC]	D1 [PC-1]	E1 [PC-2]	F1... Sample
[WCON-1] orange (colour may change)	30	30	30	30
[NC] in triplicate	70	--	--	
[PC-1]	--	70	--	
[PC-2]	--	--	70	
Sample	--	--	--	70
Orange solution changes colour to pink or yellow with the addition of sample (if sample pH is not neutral)				
Mix carefully				
[MIC] cover with adhesive protective film				
*Incubate 60 min. at 37°C				
Remove and discard the protective film slowly and carefully, prevent splashes				
Step 2				
[WCON-2] blue (add to content)	50	50	50	50
Pink solution changes colour to green				
Mix carefully				
[MIC] cover with adhesive protective film				
*Incubate 30 min. at 37°C				
Remove and discard the protective film slowly and carefully, prevent splashes				
Wash 4 times as described (see W1 – W4)				
[WASH]	400	400	400	400
Step 3				
[SUB]	100	100	100	100
Incubate 20 min. at 18...24°C (see P10)				
[STOP]	150	150	150	150
Measure the absorbance at 450 nm within 3-4 min. after terminating of the reaction, using a reference wavelength of 620 – 680 nm (if available)				
*Alternative mode using a thermo shaker, shaking with 500 rpm at 37°C Incubation time, Step 1: 45 min, Step 2: 20 min Note: Do not mix the incubation modes!				

Calculation of Control Values and Cut-off

Mean absorbance value of [NC] in wells A1, B1 and C1 (MNC) is calculated according to:

$$MNC = \frac{A_{450}\,(A1) + A_{450}\,(B1) + A_{450}\,(C1)}{3}$$

Cut-off value COV = MNC + 0.400

The test run may be considered valid provided that the following criteria are met:

1. [NC] ≤ 0.200 2. [PC-1], [PC-2] ≥ 0.800

If one [NC] ≥ 0.200, disregard and recalculate the mean value using the two remaining values. Only one value may be eliminated in this way.

Interpretation of Results

Result	Interpretation
A_{450} (specimen) < COV	HIV-Ab/HIV-Ag non-reactive
A_{450} (specimen) ≥ COV	HIV-Ab/HIV-Ag reactive

Reactive samples should be retested. If the samples are repeatedly reactive, they should be subjected to a confirmatory test (Western Blot).

Reactive result: The HUMAN HIV Ag/Ab ELISA gives no indication about the type of HIV antibody (anti-HIV-1 or anti-HIV-2) nor about detection of the HIV antigen. Further testing to distinguish between the HIV antibodies and the p24 antigen may be necessary.

Non-reactive result: Does not absolutely exclude an HIV infection due to the individual immunoreactivity or variability of the viruses or due to an anti-retroviral medication.

Performance Characteristics

HUMAN HIV Ag/Ab ELISA has been tested with confirmed HIV-1 positive samples (including subtype O), HIV-2 patient samples, patients in other clinical categories, random blood donors and commercially available seroconversion panels.

Sensitivity and Specificity

Sensitivity of 100% was determined for 504 HIV-1 positive samples including 102 identified subtypes, 132 HIV-2 positive samples and 88 samples from commercially available panels.

Seroconversion sensitivity of HUMAN HIV Ag/Ab ELISA was evaluated using 56 seroconversion panels (Seracare, ZeptoMetrix). Results were compared against commercially available, CE-marked 4th generation assays.

Diagnostic specificity has been determined to be 99.70% for unselected European donors (n=12776). For different cohorts of European blood serum samples, diagnostic specificities were calculated to be 100%: pregnant women (n=364), hospitalized patients with non-infectious diseases (n=273), patients with rheumatoid factor (n=182). In patients with infectious diseases (Hepatitis A, B, C, syphilis, chlamydiosis, herpetic and cytomegalovirus infections) (n=182) the calculated specificity is 99.45%. Diagnostic specificity for patients with a confirmed diagnosis of COVID-19 in the acute stage of the disease (n=543) was calculated to be 99.45%. No interferences were detected for biotin (up to 2.5 ng/ml), bilirubin (up to 11.7 mg/l), haemoglobin (up to 10 g/l) and triglyceride (up to 36 g/l).

Analytical sensitivity

The analytical sensitivity was determined with the 1st International Reference Reagent (NIBS code 90/636) at 1.0 IU/ml.

Precision

The CV within one plate is below 5%. The CV between plates from different lots is below 5%. The CV between different lots, operator, time of the measurement and laboratories is below 5%.

Note

- All protocols for automated analysers must be fully validated prior usage.
- Since the enzyme reaction is very sensitive to metal ions, do not allow any metal element to come into contact with conjugate or substrate solutions. Upon development of automated applications, the compatibility of any metal tips used for dispensing has to be addressed in the validation.
- As with all diagnostic tests, the results should be interpreted with due consideration of other laboratory findings and of the clinical status of the patient. A negative result does not preclude the possibility of exposure to or infection with HIV.

Safety Notes

[PC1] [PC2] Warning

H317 May cause an allergic skin reaction

H412 Harmful to aquatic life with long lasting effects

[STOP] Warning

H315 Causes skin irritation.

H319 Causes serious eye irritation.

[NC] [PC1] [PC2] [CON-1]11x] [CON-2]11x] [DIL-C1] [DIL-C2] [WS]25x] [SA]11x] [SB] [STOP]

P234 Keep only in original packaging.

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P262 Do not get in eyes, on skin, or on clothing.

P281 Use personal protective equipment as required.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337+P313 If eye irritation persists: Get medical advice/attention.

P401 Store in accordance with local/regional/national/international regulations.

P501 Dispose of contents/container in accordance with local/regional/ national/ international regulations.

References

1. Tehe A., Journal of Clinical Virology 37, 199-205 (2006)
2. Weber B., Expert Rev. Mol. Diagn. 6, 3 (2006)
3. Mahboudi E., Journal of Biotechnology 125, 295-303 (2006)
4. Stenman U. H., Clinical Chemistry 47, 815-820 (2001)
5. Niel T. *et al.*, Indian J Med Res 121, 519-538 (2005).
6. Stevens G., Journal of Clinical Microbiology 43, 857-861 (2005)
7. Tan SS. *et al*., Journal of clinical pathology **74,9**, 614 (2021)
8. Salih RQ *et al*., Annals of medicine and surgery **71:** 103027 (2021)

EL-HIV4g INF 51215 GB 08-2023-008

0483

Human Gesellschaft für Biochemica und Diagnostica mbH
Max-Planck-Ring 21 · 65205 Wiesbaden · Germany
Telefon +49 6122-9988-0 · Telefax +49 6122-9988-100 · e-Mail human@human.de