# Syphilis Screen

## ELISA for the Detection of Antibodies to *Treponema pallidum* in Human Serum and Plasma

Package Size

| | | | |
|---|---|---|---|
| [REF] | 51005 | 96 Tests | Complete Test Kit |

[IVD]

Intended Use

The HUMAN SYPHILIS SCREEN ELISA is an enzyme immunoassay for the qualitative detection of antibodies to *Treponema pallidum* in human serum or plasma. The assay is intended for professional use for screening potentially infectious samples to prevent their use as donor material, for aid in the diagnosis of patients suspected of having syphilis and for the screening of pregnant women.

Principle

The HUMAN SYPHILIS SCREEN ELISA is a two-step ELISA. Recombinant antigens (rAg) of *Treponema pallidum* are coated on the microtiter wells. The conjugate is a mixture of HRP-labeled monoclonal anti-human-IgG and anti-human-IgM antibodies (mAb). *T. pallidum* specific antibodies (anti-Tp-ab) present in serum or plasma samples bind to both the immobilised rAg and the conjugate forming double antigen-antibody complexes. After incubation unbound components are washed out and TMB Substrate is added. A blue color develops changing to yellow after stopping the reaction with sulphuric acid.

The color intensity is directly proportional to the anti-Tp-ab concentration in the specimen. The absorbance of controls and specimen is determined by using ELISA microplate readers or automated ELISA systems at 450 nm or 450/620-680 nm. HUMAN ELISA are compatible with both manual and automated applications (see “Readers and Automated Analyzers” and “Notes”).

Clinical value

Syphilis is a sexually transmitted infection (STI) caused by the bacteria *Treponema pallidum*. Syphilis is mainly acquired via sexual transmission. However, it may also be transmitted by transplacental passage to the foetus (congenital syphilis) or by blood transfusion. There are two types of serological tests for syphilis: non-treponemal (RPR, VDRL) and treponemal (TPHA, FTA-ABS, EIA). A presumptive diagnosis of syphilis requires a positive result from at least one of these types of tests. A confirmed diagnosis requires positive results from both types of serological tests.

Reagents and Contents

| | | |
|---|---|---|
| [MIC] | 12 | Microtiter Strips (in strip holder)<br>breakable 8-well strips coated with *T. pallidum* rAg |
| [NC] | 2.5 ml | Negative Control (green cap)<br>ready for use, human plasma, green dyed |
| [PC] | 2.5 ml | Positive Control (red cap)<br>ready for use, human plasma positive for antibodies to *T. pallidum*, red dyed |
| [CON]11x] | 1.2 ml | Conjugate (yellow cap)<br>mixture of HRP-labeled anti-human-IgG/IgM mAb; colorless or slightly opalescent liquid |
| [DIL-C] | 12 ml | Conjugate Diluent (transparent cap)<br>casein hydrolysate, sodium chloride, TRIS; colorless or slightly opalescent yellow liquid |
| [DIL-S] | 14 ml | Sample Diluent (transparent cap)<br>phosphate saline buffer, bovine serum albumin, TRIS; opalescent violet liquid |
| [WS]25x] | 50 ml | Wash Solution (25x) (transparent cap)<br>phosphate saline buffer; colorless or pale yellow liquid |
| [SA]11x] | 2.5 ml | Substrate A (11x) (brown cap)<br>concentrate, 3,3', 5,5'-tetramethylbenzidin (TMB) |
| [SB] | 25 ml | Substrate B (brown cap)<br>citric acid and potassium hydroxide solution, pH 4.2, containing $H_2O_2$; colorless liquid |
| [STOP] | 25 ml | Stop Solution (transparent cap)<br>sulphuric acid, ready for use, colorless 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 or thermoshaker for 37°C ± 1.0°C, 500 rpm, microplate reader equipped with 450 nm or with 450/620–680 nm filters, deionised water.

Safety Notes

All patient specimens and controls should be handled as potentially infectious. The human material used for the controls has been checked on the donor level with CE-marked tests for HBsAg, anti-HCV, anti-HIV-1/2, antigen p24 HIV-1, and found to be non-reactive. Wear protective clothing and disposable gloves according to good laboratory practices.

All materials and equipment contaminated with patient specimens or controls including [WASH] and other liquid and solid waste should be handled as infectious and should be inactivated by validated procedures (autoclaving or chemical treatment) 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 in a tightly sealed package. Once opened, the components should be used within 1 month. Concentration of preserving agents: ≤0.1 %.

- Allow to reach 18…24°C before opening!
- Lost vacuum in the bag of the coated plate will not affect the performance of the test.
- Return unused [MIC] to the resealable zip-lock bag and store it with desiccant 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]

- Dilute [WS]25x] 1 + 24 with fresh deionised water, e.g. 18 ml [WS]25x] + 432 ml deionised water for 6 strips. Mix solution thoroughly.
- Stability: 14 days at 18...24°C or 28 days at 2...8°C.

*Working conjugate solution* [WCON]

E.g., for 6 strips (100 µl required for each well):

- Dilute [CON]11x] 1 + 10 with [DIL-C], e.g. 0.6 ml [CON]11x] + 6.0 ml [DIL-C] at least 10 min before use. Mix thoroughly, avoid foaming.
- Stability: 12 hours at 18...24°C when stored at a dark place.

*Working substrate solution* [SUB]

- Dilute [SA]11x] 1 + 10 with [SB], e.g. 0.6 ml [SA]11x] + 6.0 ml [SB] for 6 strips. Mix thoroughly until diluted.
- [SUB] should be colorless. Do not use, if it appears blue!
- Stability: 10 hours at 18...24°C when stored at a dark place.

Specimens

Collect blood specimens according to the current practices. Undiluted serum and plasma with the anticoagulants citrate, heparin or EDTA can be used. Do not use heated specimens. Do not use pooled samples. Do not use contaminated, hyperlipaemic and hyperhaemolysed specimens. Samples with hyperproteinaemia and hyperbilirubinaemia were not tested. To avoid haemolysis, separate the clot or red cells from serum or plasma as soon as possible. Specimens may be stored for 2 days at 2...8°C or longer at -20°C. Freeze and thaw only once. Particulate matter should be eliminated by centrifugation. Suspended fibrin particles or aggregates may yield reactive results.

Procedure

Follow the procedure exactly as described.

*Procedural Notes*

P1: Do not mix reagents from different lot numbers within a run. Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number of microwells.

P5: Do not let the wells dry once the assay has been started.

P6: Do not reuse the coated plates.

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

P8: Do not reuse the removed protective film.

P9: Always add reagents in the same order and timing, according to the assay procedure and without interruptions, to minimise reaction time differences between wells. This is important for reproducible results.

P10: Avoid/remove air bubbles prior to incubation and reading of absorbance.

P11: Do not expose the reagents to excessive heat or sunlight during storage and test procedure.

P12: Do not use reagents without label or with damaged label / package.

P13: Always firmly close vials with the proper caps after use.

P14: Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc.

P15: Always store stock solutions at 2...8°C when not in use; do not freeze the reagents.

P16: Do not run the test in the presence of reactive vapours (acid, alkaline, aldehyde), dust or metals.

*Wash Procedure*

The wash procedure is critical. Insufficient washing will result in poor precision. The use of an automatic washer is strongly recommended.

W1: Remove adhesive protective film, aspirate off the contents and add [WASH] to each well using flow-through washing with a volume of at least 400 µl per well. If not possible, ensure that the well is completely filled with a slight positive meniscus without overflow. Aspirate off after 40 sec. soak time and repeat washing 4 times.

W2: Ensure that no liquid is left in the well (use double aspiration in the final step where possible). Avoid tapping the plate upside down.

W3: When using a microplate washer, clean the wash head frequently to prevent contamination. Residual volume lower than 10 µl is not critical for the assay.

W4: Do not allow the microwells to dry during the assay procedure.

*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 [PC] | B1/C1/D1 [NC] | E1... Specimen | |
| [PC] | 100 | | | |
| [NC] in triplicate | | 100 | | |
| [DIL-S] | | | 90 | |
| Specimen | | | 10 | |
| [MIC] cover with adhesive protecting film | | | | |
| *[1] Incubate 30 min. at 37°C | | | | |
| Wash 4 times as described (see W1 – W4) | | | | |
| *Step 2* | | | | |
| [WCON] | 100 | 100 | 100 | 100 |
| [MIC] cover with adhesive strips | | | | |
| *[2] Incubate 30 min. at 37°C | | | | |
| Wash 4 times as described (see W1 – W4) | | | | |
| *Step 3* | | | | |
| [SUB] | 100 | 100 | 100 | 100 |
| Incubate 20 min. at 18...24°C in the dark | | | | |
| [STOP] | 150 | 150 | 150 | 150 |
| Measure the absorbance at 450 nm within 3 min. after terminating the reaction, using a reference wavelength of 620 - 680 nm (if available). | | | | |
| *Alternative mode:<br>*[1] Incubate in microplate thermoshaker for 15 minutes at 500 rpm at 37.0 ± 1.0 °C.<br>*[2] Incubate in microplate thermoshaker for 20 minutes at 500 rpm at 37.0 ± 1.0 °C.<br>Note: Do not mix the incubation modes! | | | | |

Readers and Automated Analyzers

Validated settings for HUMAN ELISA microplate readers (HumaReader) or automated HUMAN ELISA analyzers (ELISYS line) are preinstalled or can be obtained from your local distributor. For automated analyzers, other than those provided by HUMAN, follow section Pipetting Scheme and ensure all requirements described in section Procedural Notes are followed. All protocols for automated analyzers must be fully validated before usage.

Calculation of Control Values and Cut-off

Mean absorbance values of [NC] in wells B1, C1 and D1 (MNC) are calculated according to:

$$MNC = \frac{A_{450}(B1) + A_{450}(C1) + A_{450}(D1)}{3}$$

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

1. [PC] >0.600
2. [NC] < 0.200

If one [NC] is out of limit, the value can be excluded from the calculation of MNC. If more than one [NC] is out of limit, the run should be repeated.

Cut-off value COV = MNC + 0.350

Interpretation of Results

| Result | Interpretation |
|---|---|
| $A_{450}$ (specimen) ≤ 0.9 COV | non-reactive |
| $A_{450}$ (specimen) ≥ 1.1 COV | reactive |
| 0.9 COV < $A_{450}$ (specimen) < 1.1 COV | repeat test after 1-2 weeks with fresh sample |

If the sample falls in the gray area (0.9 COV < $A_{450}$ (specimen) < 1.1 COV), it is advisable to test the sera samples simultaneously with the previous ones (pair samples), it will allow to assess specific antibody dynamics more accurately.

Performance Characteristics

HUMAN SYPHILIS SCREEN ELISA has been verified by testing samples from random blood donors, patients with confirmed syphilis infection and patients in other clinical categories.

*Sensitivity and Specificity*

HUMAN SYPHILIS SCREEN ELISA has been tested with 401 Anti-*Treponema pallidum* antibodies positive samples including (sensitivity in parenthesis) 32 patients with primary stage of syphilis (96.90%), 93 patients with secondary stage of syphilis (100%), 159 patients with early latent stage of syphilis (100%), 24 patients with latent stage of syphilis (100%), 93 patients with known past syphilitic infection (100%) for a combined sensitivity of 99.75%. Additionally, the following panels have been tested (number of samples and sensitivity in parenthesis): BBI PSS202 mixed titer performance panel (20 samples, 100%), BBI QSS701 qualification panel (6 samples, 100%), Zeptometrix K-ZMC002, mixed titer performance panel (15 samples, 100%).

HUMAN SYPHILIS SCREEN ELISA has been tested with (diagnostic specificity in parenthesis) 5012 unselected blood donors (99.78%), 206 hospitalized patients with non-infectious diseases (99.03%), 40 pregnant women (100%), 71 patients with rheumatoid factor(94.37%), 29 patients with infectious diseases (HIV) (93.10%).

*Analytical Sensitivity*

The analytical sensitivity was evaluated with WHO International Standard 1st IS for human syphilitic plasma IgG (NIBSC Code: 05/122) and defined at 0.026 IU/ml.

*Precision*

The CV within one plate was evaluated by testing 3 positive samples 24 times each. The CV did not exceed 6 %. The CV between plates was evaluated by testing 3 positive samples 48 times each. The CV did not exceed 7 %. The CV between different lots, operators, days was evaluated by testing 3 positive samples 72 times. The CV did not exceed 11 %.

Notes

1. All protocols for automated analyzers must be fully validated prior usage.
2. 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 *Treponema pallidum*.
3. Non-reactive results can occur if the concentration of marker present in the sample is below the detection limit of the assay, or if the marker to be detected is not present during the stage of disease when a sample has been collected.
4. A sample should not be defined as positive for anti-*Treponema pallidum* antibodies based on a single reactive result. Reactive results should be re-tested; and in case of repeated reactive result confirmed by supplemental assays.
5. False positive results may be observed in patients with HIV infection, virus hepatitis, cancer, chlamidiosis, pregnancy, infectious mononucleosis, leprosy, autoimmune diseases and drug addiction.

Safety Notes

~~[SA]11x] Danger~~

~~H315 Causes skin irritation.~~

~~H317 May cause an allergic skin reaction.~~

~~H319 Causes serious eye irritation.~~

~~H360D May damage the unborn child.~~

~~H335 May cause respiratory irritation.~~

[STOP] Warning

H315 Causes skin irritation.

H319 Causes serious eye irritation.

[NC] [PC] [CON]11x] [DIL-C] [DIL-S] [WS]25x] [SA]11x] [SB] [STOP]

P234 Keep only in original container.

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.

The controls have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative.

References


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EL- SyphS INF 5100501 GB 06-2020-008



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