Version 05-09/25

User's Manual

Candida albicans antigen ELISA Kit

REF **DEIA809**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

Candida albicans antigen ELISA Kit is a quantitative and qualitative immunoassay for the detection of Candida antigen in human serum or plasma. The assay is recommended for the detection of systemic candidosis.

General Description

Candida albicans is an ubiquitous yeast which, like all Candida species, belongs to the family of yeast-like fungi. Apart from the yeast form which primarily causes superficial infections, so called pseudo mycelia are a further morphologic manifestation of yeast-like fungi. Germ tubes and the development of pseudo mycelia mainly occur in cases of systemic mycosis. Candida ssp. produce and excrete a range of destructive enzymes that enable the facultative pathogen microorganisms to penetrate mucous membrane barriers and blood vessels barriers.

Candida ssp. are primarily transmitted by smear contamination from person to person. The primary portal of entry is the oral cavity. Changes in the fungistatic properties of the skin, which are a consequence of a slightly acidic pH value and the antagonistic bacterial flora, can facilitate the establishment of superficial candidiasis of the skin surface. Systemic mycosis results from colonization of mucous membranes, particularly in the gastrointestinal tract.

Candida ssp. are able to adhere to the epithelia of a variety of mucosal membranes by adherence proteins and other cell surface structures. The schematic below shows hypothetical steps that may lead to disseminated infections in cases of severe underlying conditions. An exact description of the various stages is not practical (Fig. 1).

Candidiasis can generally be classified into two major groups whose main characteristics are listed in the table below.

The diagnosis of candidiasis on the basis of serological methods is not straight forward: On the one hand transient yeast colonization may induce an antibody response, on the other hand systemic Candida mycosis in immunosuppressed patients may only lead to minor changes in antibody activities. Such situations make critical interpretation of serological findings necessary. In addition, systemic Candida-infections may not cause typical symptoms.

Currently it is not possible to conclude that the results of different test systems for anti-Candida antibody detection are comparable or even exchangeable. Specificity of detected antibodies significantly depends on the test system (ELISA or HAT) or on the antigen preparation used. The detection of IgM antibodies with HAT is better than the IgG detection due to higher agglutination properties of IgM molecules. HAT mainly detects antibodies against cell wall antigens of yeast-like fungi which makes serological interpretation even more complex but gives the opportunity for differentiation. Changes in antibody concentrations may be detectable with ELISA but not with HAT, making a combination of the two techniques advantageous, e.g. in case of decreasing IgM antibody activity and simultaneously increasing IgG antibody activity.

Currently no single technique in isolation allows for a definitive serological diagnosis of candidiasis. Surveillance of at risk patients and therapy control requires the use of a variety of methods including serology and antigen detection. The Candida albicans antigen ELISA Kit is of particular help in such situations by detecting Candida specific antigen in patient samples.

Principles of Testing

The ELISA (Enzyme Linked Immunosorbent Assay) is an immunoassay, which is particularly suited to the determination of antibodies and antigens in the field of infectious serology. The reaction is based on the specific interaction of antibodies with their corresponding antigen. The test strips of the Candida albicans ELISA antigen microtiter plate are coated with specific antibodies directed against the pathogen of interest. If antigens in the patient´s sample are present, they bind to the fixed antibody. A secondary antibody, which has been conjugated with the enzyme peroxidase, detects and binds to the immune complex. The colourless substrate hydrogen peroxide (H2O2) and the chromogen tetramethylbenzidine (TMB) are converted into a blue coloured product. Addition of stopping solution changes the colour to yellow. The signal intensity of this reaction product is proportional to the concentration of the antigen in the sample and is measured photometrically.

Reagents And Materials Provided

1. Break apart microtiter test strips each with eight antibody coated single wells,(altogether 96) MTP, 1 frame, 12 pieces.
2. Standard serum STD, 3x3 ml.
3. Negative control serum NEG, 2x3 ml.
4. Anti-Candida albicans conjugate (ready-to-use) PODCH, 13 ml.
5. Washing solution concentrate (sufficient for 1500 ml) WASH, 2x25 ml.
6. Dilution buffer SAMB, 15 ml.
7. Stopping solution STOP, 13 ml.
8. TMB-substrate solution (ready-to-use) TMB, 13 ml.
9. Quality control certificate with standard curve and evaluation table INFO, 2 pieces.

Materials Required But Not Supplied

1. Common laboratory equipment
2. Photometer for microtiter plates with filter, wavelength 450 nm,

recommended reference wavelength 620 nm-690 nm (e.g. 650 nm)

3. Incubator 37 °C
4. Heating block 110 °C
5. Moist chamber
6. Distilled water
7. Click-Clips

Storage

This kit should be stored at 2-8 °C.

Specimen Collection And Preparation

Lipaemic, hemolytic or icteric samples (serum or plasma) should only be tested with caution. Obviously contaminated samples should not be tested. Serum or plasma (EDTA,citrate, heparin) collected according to standard laboratory methods are suitable samples.

(1) Sample Preparation

Before running the test, specimens (patient samples, standard and negative control) (V1) must be diluted in dilution buffer (V2) as follows:

V1 + V2 = 3+1

add 300 µl sample (V1) each to 100 µl dilution buffer (V2)

After dilution and before pipetting into the microtiter plate, the samples must be mixed thoroughly to prepare a homogenous solution.

Finally, the samples must be heated at 110 °C (+/- 5 °C) for 10 minutes. It is essential to keep both the time and temperature stated! Standard reagent vessels are not suitable for this thermolysis step and we recommend the use of safe-lock reaction vessels or similar vessels with a screw closure.

Usage of a heating block thermostat requires controlling of the actual temperature by a calibrated thermometer. Consider the device-specific pre-heating period of the heating block. If necessary, the heating block must be adjusted to the required temperature. During thermolysis, a white cloudy precipitate will form and should be removed by centrifugation in a pre-cooled (4 °C) table top centrifuge at 10,000 × g for 10 minutes. The clear supernatant can then be used in the test.

(2) Sample Storage

The treated patient's samples should not be stored for more than 24 hours at 2 – 8 °C. Untreated samples should not be stored at 2 – 8 °C for more than 7 days. Extended storage is possible at ≤ -20 °C. Avoid repeated freezing and thawing of samples.

Reagent Preparation

Bring all reagents to room temperature before testing.

(1)Microtiter Test Strips

The microtiter test strips in frames are packed with a desiccant in an aluminum bag. Take unrequired cavities out of the frame and put them back into the aluminum bag. Close bag carefully to ensure airtight conditions.

(2)Control Sera/Standard Sera

Control and standard sera must be diluted with sample buffer and subsequently denatured. For each test run – independent of the number of microtiter test strips to be used – control and standard sera must be included. The standard sera should be set up in duplicate.

(3)Conjugate (ready-to-use)

Avoid contamination of the ready-to-use conjugate, e.g. by using sterile tips.

(4)Wash solution

Dilute washing buffer concentrate (V1) 1:30 with aqua dest. to a final volume of V2. Example: Buffer

concentrate (V1) 25 ml add to Final volume (V2) 750 ml, or Buffer concentrate (V1) 1 ml add to Final volume (V2) 30 ml.

(5)TMB Substrate (ready-to-use)

Avoid contamination of the ready-to-use substrate solution, e.g. by using sterile tips. The TMB substrate is coloured light blue-green and any solutions which exhibit a strong colour (extinction at 650 nm against distilled water of>0.2 OD) should be discarded.

(6)Stopping Solution (ready-to-use)

Assay Procedure

1. Pretreatment of samples (patient samples, standard and control serum) as described.
2. Place the required number of cavities in the frame and prepare a protocol sheet.
3. Add each 100 µl of the supernatants from the standard serum (in duplicate), negative control and patient samples into the appropriate wells of microtiter test strips. Spare one well for substrate blank, e.g.:

Candida *antigen* quantitative	
cavity A1	Substrate blank
cavity B1	Negative control
cavity C1	Standard serum
cavity D1	Standard serum
cavity E1	Patient sample 1....

4. Sample incubation for 60 minutes (+/- 3 min.) at 37 °C (+/- 1 °C) in moist chamber.
5. After incubation wash all wells with washing solution (by automated washer or manually):

(1)aspirate or shake out the incubation solution

(2)fill each well with 300 µl washing solution

(3)aspirate or shake out the washing buffer

(4)repeat the washing procedure 4 times (altogether 5 times!)

(5)dry by tapping the microtiter plate on a paper towel

6. Addition of conjugate

Add 100 µl of the ready-to-use conjugate into the appropriate wells (except substrate blank).

7. Conjugate incubation for 60 minutes (+/- 3 min.) at 37 °C (+/- 1 °C) in moist chamber.
8. After incubation wash all wells with washing solution (see above).
9. Addition of substrate

Add 100 µl of ready-to-use TMB-substrate solution into each well (including well for substrate blank!).

10. Substrate incubation for 30 minutes (+/- 1 min.) at 37 °C (+/- 1 °C), protected from light in moist chamber.
11. Stopping of the reaction

Add 100 µl stopping solution into each well. Agitate the microtiter plate gently to mix.

12. Read extinction

Read optical density (OD) within 60 minutes at 450 nm against substrate blank, reference wavelength between 620 nm and 690 nm (e.g. 650 nm).

Notes:

(1) CD ELISA are suited for processing on automats and evaluated for use with ImmunomatTM and Gemini. The automated processing is performed analogous to manual use. Please note, that under special working-conditions internal laboratory adaptations of the incubation times may be necessary.

(2) For the periodic verification of the test method, in order to fulfil the requirements of laboratory internal quality management systems, we recommend using the kit controls to determine precision and accuracy of ELISA antigen test runs. The use of the kit controls is described in specific instruction manuals.

Interpretation Of Results

Positive test results for patient samples indicate a Candida fungemia. Results can only be interpreted in combination with the clinical picture and other detection methods.

A result can be considered as positive when at least 2.6 units are measured in the test. Such a result indicates that the sample contains the quantity of Mannan that is associated with 2.6 ng of Candida protein. However, it cannot distinguish between live or dead organisms, respectively.

Negative test results do not exclude an acute infection, especially if high antibody titers are found. In such cases it may be that the antigen is completely masked by antibodies and even the denaturing procedure during sample preparation is not sufficient to allow detection of the antigen. In addition, the point in time during an infection when a sample is obtained, an unsuitable sample, poor sample preparation and storage can all lead to a negative result.

Evaluation

Optimised assignment of extinction signals to quantitative values is guaranteed by using non-linear functions, which adjust a sigmoide curve without any further transformation to OD-values. Determination of antigen concentrations with the ELISA antigen is carried out by the 4 parameter logistic-log-model (4 PL) which is ideal for exact curvefitting. It is based on the formula:

$$OD = A + \frac{D - A}{1 + e^{B(C - \ln Conc.)}}$$

A: lower asymptote

B: slope of the curve

C: turning point

D: upper asymptote

For each lot the standard curve is evaluated by Creative Diagnostics in repeated test runs under optimal conditions. Time consuming and cost intensive construction of the standard curve by the user is not necessary.

For evaluation of antigen concentrations a lot specific standard curve as well as a lot specific evaluation table is included with each ELISA antigen test kit. The evaluation software CD evaluate as well as the Microsoft® Excel-based software tool CD activity are available on request.

To compensate for normal test variations and also for test run control a standard serum is used in each individual test run. For this control serum a reference value with a validity range is determined by the quality control of the producer. Within this range a correct quantification of antigen concentration is ensured.

Detection Range

The borderline range of the ELISA antigen Candida test is specified on the quality control certificate and indicates the range for borderline test results. Values obtained, when testing a patient's sample, which fall below this range indicate a negative test result; values above the borderline range are interpreted positive. In cases where the results are within the borderline range a definitive interpretation of the result is not possible. In such cases, the test should be repeated in parallel with a follow-up sample taken one to two weeks later (serum pair).

Detection Limit

The limits of quantification are specified on the quality control certificate of the ELISA antigen test. The linearity of dilution within this range has been demonstrated in comprehensive evaluation studies. In case a patient sample shows a test result above the upper limit of quantification, the sample may be tested at a higher dilution. The thereby determined antigen activity must be multiplied by the additional dilution factor.

Sensitivity

The ELISA antigen Candida test was validated by the analysis of 148 serum samples from blood donors and 93 specimens from patients, who showed signs of candidiasis during intensive care treatment, in comparison to the results obtained with a commercially avaliable assay of a leading European manufacturer. Sera classified as borderline were not included in the calculation of sensitivity and specificity values.

Performance Characteristics	Sensitivity	Specificity
ELISA antigen Candida	>99.9 %	97.8%

In another internal study, cross-reactivity with the species Candida guillermondii, Candida glabrata, Candida krusei, Candida parapsilosis and Candida tropicalis with the ELISA antigen Candida was demonstrated in order to guarantee a comprehensive Candida diagnosis.

Due to a similiarity of mannan with hydroxyethylstarch (HES), which is used in plasma expanders for treatment of circulatory failure (hypovolemic, hemorrhagic and septic shock), potential cross-reactivity was assessed. However, no cross-reaction with HES was measureable when using the ELISA antigen Candida test.

Reproducibility

Intraassay reproducibility was determined by testing sera of different reactivities 20 times in one test run. Interassay reproducibility was determined by testing sera of different reactivities in 10 independent test runs.

$$\text{Coefficient of Variation (CV \%)} = \frac{\text{Standard deviation}}{\text{Mean value}} \times 100$$

CD ELISA antigen Candida:

Sample	Mean Value (OD)	Intraassay (CV %)	Mean Value (OD)	Interassay (CV %)
negative	0.223	9.6	0.225	7.3
borderline	0.507	2.4	0.476	5.3
positive	1.413	2.5	1.356	2.7
positive	2.851	2.0	2.442	8.1

Precautions

1. Only use the kit reagents when using Candida albicans antigen ELISA Kit. The components must not be exchanged for reagents of other manufacturers. Standard and control sera as well as the conjugate are defined exclusively for the test kit to be used and must not be used in other lots. Dilution buffer, washing solution, substrate and stop solution can be used for all Candida albicans antigen ELISA Kit irrespective of the lot and the test.
2. Unopened, all components of the kit may, if stored accordingly, be used up to the expiry dates given on the labels. Reagents may not be used after date of expiry. Dilution or alteration of the reagents may result in a loss of sensitivity.
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4. Avoid exposure of reagents to strong light during storage and incubation. Reagents must be tightly closed after use to avoid evaporation and contamination.
5. To open the aluminium bag of the microtiter plate please cut off the top of the marked side only, in order to guarantee proper resealing. Do not use the strips if the aluminium bag is damaged or if the bag with remaining strips and desiccant was not properly resealed.
6. Use aseptic techniques when removing aliquots from the reagent tubes to avoid contamination. To avoid false positive results ensure not to contact or splash the top-walls of wells while pipetting conjugate. Take care not to mix the caps of the bottles and/or vials.
7. In particular, the TMB-substrate solution is sensitive to oxidising substances and heavy metal ions. It must be stored away from light at all times and only opened in low light conditions immediately prior to use. This solution is coloured light blue-green.
8. Skin contact with substrate and stop solution should be avoided.
9. Reproducibility of test results is dependent on thorough mixing of the reagents. Agitate the flasks containing control sera before use and also all samples after dilution (e.g. by using a vortex mixer).
10. Be sure to pipette carefully and comply with the given incubation times and temperatures. Significant time differences between pipetting the first and last well of the microtiter plate when dispensing samples and control sera, conjugate or substrate can result in different pre-incubation times, which may influence the precision and reproducibility of the results.
11. Optimum results can only be achieved if the instructions are strictly followed.
12. The ELISA antigen immunoassay is only valid if the lot-specific validation criteria on the quality control certificate are fulfilled.

13. Adequate washing avoids test unspecificities. Therefore, the washing procedure should be carried out carefully. All of the flat bottom wells should be filled with equal volumes of washing buffer. At the end of the procedure ensure that the wells are free of all washing buffer in order to avoid uncontrolled dilution effects. Avoid foaming!
14. Take care not to damage the inscription (pathogen / AG) on the microtiter test strips during washing and aspiration to avoid confusion.