SERION ELISA *antigen* Candida

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Enzyme Immunoassay for detection of Candida Antigen

- For sale in the U.S. for Research Use Only. Not for use in diagnostic procedures.

SERION ELISA *antigen* Candida order number: ESR200

1 INTENDED USE

SERION ELISA *antigen* Candida is a quantitative and qualitative test for detection of *Candida* antigen in laboratory samples of human serum or plasma.

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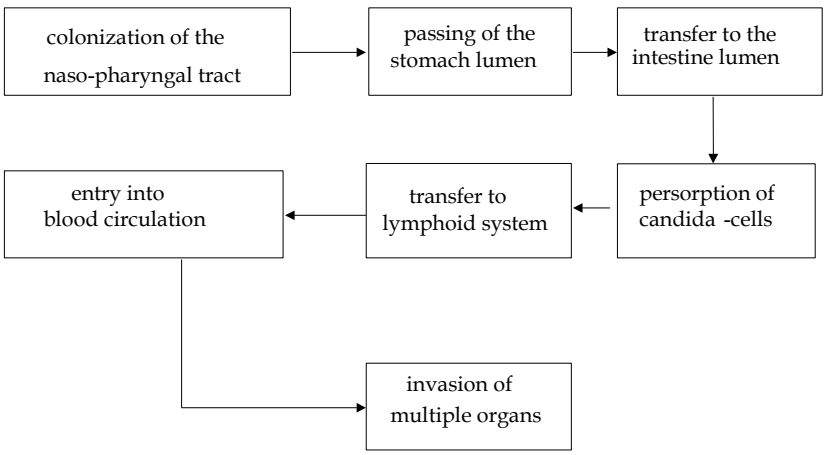
2 BACKGROUND

Candida albicans is a ubiquitous yeast that belongs to the family of yeastlike fungi. Apart from the yeast form which primarily causes superficial infections, so called pseudomycelia are a further morphologic manifestation of yeastlike fungi. Germ tubes and the development of pseudo mycelia mainly occur in cases of systemic mycosis. *Candida* yeastlike fungi produce and excrete a range of destructive enzymes that enable this organism to penetrate blood vessels and mucosa 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 and the antagonistic bacterial flora, can facilitate the establishment of superficial candidiasis of the skin surface. Systemic mycosis results from colonization of mucus membranes, particularly in the gastrointestinal tract.

Candida spp. are able to adhere to the epithelia of mucosal membranes by adherence proteins and other cell surface structures. The following scheme shows hypothetical steps that may lead to disseminated infections in severe cases. An exact discrimination of the stages is not practical .



Candida ssp. are not part of the physiological microflora of healthy humans. However, repeated colonization of the human host is frequently observed.

3 SERION ELISA *antigen* - TEST PRINCIPLE

The test strips of the SERION ELISA *antigen* microtiter plate are coated with specific antibodies directed against the organism of interest. If antigens in a sample are present, they bind to the coated antibody. A second antibody which has been conjugated with the enzyme peroxidase detects and binds to the antibody-antigen complex. The colorless substrate hydrogen peroxide (H_2O_2) and the chromogen tetramethylbenzidine (TMB) are converted into a blue colored product. Addition of stopping solution changes the color to yellow. The signal intensity of this reaction product is proportional to the concentration of the antigen in the sample and is measured photometrically.

4 KIT COMPONENTS

Test components	amount / volume
Break apart microtiter test strips each with 8 antibody coated single wells (altogether 96) MTP**,** 1 frame	12 strips
Standard serum STD *Candida albicans* antigen in human serum supplemented with fetal bovine serum (FBS); negative for anti-HIV antibody, HBs-Ag (Hepatitis B-Virus surface antigen) and anti-HCV antibody; preservative <0.1% sodium azide.	3 x 3ml
Negative control serum NEG Human serum supplemented with fetal bovine serum (FBS); negative for anti-HIV antibody, HBs-Ag (Hepatitis B-Virus surface antigen) and anti-HCV antibody; preservative <0.1% sodium azide	2 x 3ml
Anti-*Candida albicans* conjugate (ready-to-use) PODCR Anti-*Candida albicans* antibody conjugated to horseradish peroxidase (HRP); stabilized with protein stabilization solution containing detergent; preservative 0.1% ProClin® 300.	13ml
Washing solution concentrate (sufficient for 1500ml) WASH TRIS-buffered salt solution with Tween 20; 30-fold concentrated; preservative <0.1% ProClin® 300	2 x 25ml
Dilution buffer SAMB Acid solution without preservative	15ml
Stopping solution STOP 0.5N sulfuric acid	13ml
TMB-substrate solution (ready-to-use) TMB TMB-hydrogen peroxide substrate solution; preservative <0.01% Kathon CG	13 ml
Quality control certificate with standard curve and evaluation table INFO (quantification of antigens in IU/ml or U/ml)	2 pieces

5 MATERIAL REQUIRED BUT NOT SUPPLIED

- common laboratory equipment
- photometer for microtiter plates with filter, wavelength 450 nm, recommended reference wavelength 620 nm - 690 nm (e.g. 650 nm)
- incubator 37°C
- heating block 110°C
- moist chamber
- distilled water

6 STORAGE AND STABILITY

Reagent	Storage	Stability
Microtiter strips (coated with antibody)	Unopened After opening at 2-8°C in closed aluminum bag with desiccant *Strips which are not used must be stored dry and air tight in the closed aluminum bag.*	see expiry date minimum shelf-life 4 weeks shelf-life in case of proper use and storage until expiry date
Control sera / Standard sera	After opening at 2-8°C	until expiry date; 24 months from date of production
Conjugate	Ready-to-use solution, at 2-8°C *Avoid contamination by using sterile tips.*	until expiry date; 18 months from date of production
Dilution buffer	Unopened After opening at 2-8oC *Discard cloudy solutions!*	until expiry date; 24 months from date of production 6 months
Washing solution	Concentrate after opening at 2-8°C Working dilution at 2-8°C Working dilution at room temperature *Bottles used for the working dilution should be cleaned regularly, discard cloudy solutions.*	until expiry date; 24 months from date of production 2 weeks 1 week
TMB substrate	Ready-to-use solution at 2-8°C, protected from light! *Avoid contamination by using sterile tips. Discard if solution turns blue (extinction against distilled water at 650nm > 0.2 OD).*	until expiry date 24 months from date of production
Stopping solution	After opening at room temperature	until expiry date

7 TEST PROCEDURE SERION ELISA *antigen*

7.1 General Notes

- Only use SERION ELISA *antigen* reagents for test procedure. The components must not be exchanged for reagents of other manufacturers. Standard and control sera as well as the conjugate of SERION ELISA *antigen* immunoassays are defined exclusively for the test kit to be used and must not be used with other lots. Dilution buffer, washing solution, substrate and stop solution can be used for all SERION ELISA *antigen* immunoassays irrespective of the lot and the test.
- Unopened, all components of the SERION ELISA *antigen* tests may be used up to the expiry dates given on the labels if stored according to instructions. Reagents should not be used after date of expiry.
- Dilution or alteration of the reagents may result in a loss of sensitivity.
- Avoid exposure of reagents to strong light during storage and incubation. Reagents must be tightly closed after use to avoid evaporation and/or contamination.
- To open the aluminum 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 aluminum bag is damaged or if the bag with remaining strips and desiccant was not properly resealed.
- Use aseptic technique when removing aliquots from the reagent tubes to avoid contamination. To avoid false positive results, be careful not to contact or splash the tops of wells while pipetting conjugate. Take care not to mix the caps of the bottles and/or vials.
- In particular, the TMB substrate solution is sensitive to oxidizing 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 light blue-green in color.
- Avoid skin contact with substrate and stop solution.
- Reproducibility of test results is dependent on thorough mixing of the reagents. Gently mix the tubes of control sera and diluted samples before use with a vortex mixer.
- 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 ser, conjugate, or substrate can result in different pre-incubation times which may influence the precision and reproducibility of the results.
- Optimum results can only be achieved if the instructions are strictly followed.
- The SERION ELISA antigen immunoassay is only valid if the lot-specific validation criteria on the quality control certificate are fulfilled.
- Adequate washing eliminates non-specific reactions, therefore, the washing procedure should be performed carefully. All of the microtiter wells should be filled with equal volumes of wash buffer. At the end of the procedure, ensure that the wells are free of all wash buffer in order to avoid dilution effects. Avoid foaming!
- Be careful not to damage the labeling on the microtiter test strips during washing and aspirating to avoid confusion.

7.2 Sample Preparation and Storage

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

7.2.1 Sample Preparation

Before running the test, samples (V_1) must be diluted in dilution buffer (V_2) as follows:

$V_1 + V_2 = 3 + 1$	add	300µl	sample (V_1)
	each to	100µl	dilution buffer (V_2)

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 important not to increase or decrease this temperature and time. Use heat-resistant screw-cap tubes.

If a white cloudy precipitate forms as a result of the heat treatment, remove it by centrifugation in a pre-cooled (4°C) centrifuge at 10,000 x g for 10 minutes. The clear supernatant can then be used in the test.

7.2.2 Sample Storage

Heat-treated 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.

7.3 Preparation of Kit Reagents

Bring all reagents to room temperature before use.

7.3.1 Microtiter Test Strips

Microtiter test strips in frame are packed with desiccant in an aluminum bag. Take unrequired wells out of the frame and put them back in the aluminum bag. Close bag carefully to ensure airtight conditions.

7.3.2 Control Sera / Standard Sera

Control and standard sera must be diluted with sample buffer and subsequently heat-treated as above. 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.

7.3.3 Conjugate (ready-to-use)

Avoid contamination of ready-to-use conjugates by using sterile pipette tips.

7.3.4 Wash Solution

Dilute wash buffer concentrate (V_1) 1:30 with distilled water to a final volume of V_2.

Example:

Wash buffer concentrate (V_1)	Final volume (V_2)
25ml	750ml
1ml	30ml

7.3.5 TMB Substrate (ready-to-use)

Avoid contamination of the ready-to-use substrate solution by using sterile pipette tips. The TMB substrate should be light blue-green in color before use. Discard if solution becomes darker blue.

7.3.6 Stopping Solution (ready-to-use)

7.4 Overview - test procedure

SERION ELISA *antigen* Candida

quantitative

Preparation of samples, control, and standard serum

300ul Sample + 100ul Dilution Buffer
Incubate 10 min. @ 110°C
Centrifuge 10 min. / 10,000 x g @ 4°C
Retain supernatant, discard pellet

⇩

Add supernatant from samples (100ul/well)

⇩

INCUBATION 60 min. @ 37°C
moist chamber

⇩

WASH (5 x 300µl DIL WASH) [1]

⇩

Pipette conjugate solution PODCR (100 µl)

⇩

INCUBATION 60 min. @ 37°C
moist chamber

⇩

WASH (5 x 300µl DIL WASH) [1]

⇩

Pipette substrate solution TMB (100 µl)

⇩

INCUBATION 30 min. @ 37°C
moist chamber

⇩

Pipette stopping solution STOP (100 µl)

⇩

READ EXTINCTION AT 450nm (ref. 650nm)

[1]For manual use: at the end of wash procedure tap plate on paper towel

7.5 ManualTest Procedure

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

Candida *antigen* quantitative	
well A1	Substrate blank
well B1	Negative control
well C1	Standard serum
well D1	Standard serum
well E1	Test 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 wash solution (by automated washer or manually):
   - aspirate or shake out the incubation solution
   - fill each well with 300µl wash solution
   - aspirate or shake out the wash buffer
   - repeat the washing procedure 4 times (altogether 5 times!)
   - dry by tapping the microtiter plate on a paper towel
6. **Addition of conjugate**
   Add **100 µl of the ready-to-use conjugate** to the appropriate wells (except substrate blank).
7. **Conjugate incubation** for 60 minutes (+/- 1 min) * at 37°C (+/- 1°C) in moist chamber.
8. After incubation **wash** all wells with wash solution (see above).
9. **Addition of substrate**
   Add **100µl ready-to use TMB substrate solution** to 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 the reaction**
    Add **100µl stop solution** to each well. Agitate the microtiter plate gently to mix.
12. **Read optical density**
    Read OD within 60 minutes at 450nm against substrate blank, reference wave length between 620 nm and 690 nm (e.g. 650 nm).

7.6 Automated Test Procedure

SERION ELISA are suited for processing on automats and evaluated for use with Immunomat™ 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.

7.7 Positive Control / Accuracy Control

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

8 TEST EVALUATION

8.1 Single-point quantification with the 4PL method

Optimized 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 SERION ELISA *antigen* is carried out by the **logistic-log-model (4 PL**; **4 parameter)** which is ideal for exact curve-fitting. It is based on the formula:

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

The parameters A, B, C, and D are representative for the exact shape of the curve:

1. lower asymptote ⇨ parameter A
2. slope of the curve ⇨ parameter B
3. turning point ⇨ parameter C
4. upper asymptote ⇨ parameter D

For each lot the standard curve is evaluated by Institut Virion\Serion GmbH (Würzburg, Germany) in several 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 test kit. Appropriate evaluation software is 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 manufacturer. Within this range a correct quantification of antibody concentration is ensured.

8.2 Criteria of validity

- The substrate blank must be OD < 0.25.
- The negative control must produce a negative test result.
- Quantitative ELISA: the mean OD-value (after subtraction of the substrate blank)of the standard serum must be within the validity range which is given on the lot specific quality control certificate of the kit
- The variation of OD values of the standard serum may not be higher than 20%.

If these criteria are not met, the test is not valid and must be repeated.

8.3 Calculation SERION ELISA *antigen* Candida

8.3.1 Non-automated evaluation

For the test evaluation a lot-specific quality control certificate with standard curve and an evaluation table is included in the test kit. The substrate blank must be substracted from all OD values prior to evaluation.

Method 1: Qualitative Evaluation

To fix the cut-off ranges multiply the mean value of the measured standard OD with the numerical data of the quality control certificate (see special case formulas), e.g.:

OD = 0.502 x MW (STD) with upper cut-off

OD = 0.352 x MW (STD) with lower cut-off

If the measured mean absorbance value of the standard serum is 0.970, the range of the cut-off is in between 0.341-0.487 OD.

Method 2: Continuous determination of antigen activities using the standard curve.

So called *interassay variations* (day to day deviations and laboratory to laboratory deviations) are compensated for by multiplication of the current measured value obtained with a sample with the **correction factor F**. This factor is calculated as follows:

$$F = \frac{\text{OD-reference value (of standard serum)}}{\text{OD-current value (of standard serums)}}$$

The procedure is necessary to adjust the current level of the test of the user with the lot-specific standard curve. First, daily deviations have to be corrected by calculating a factor (correction factor F):

1. The mean of the two OD values of the standard serum has to be calculated and checked that it is within the given validity range.

2. Calculation of the factor "F": the given reference value is divided by the mean of the extinction of the standard serum:
   F = reference value extinction STD serum / mean value extinction STD serum.

3. All measured values of test samples are multiplied by "F".

4. Antigen activities in IU/ml or U/ml can be determined from the standard curve with the corrected values.

8.3.2 Automatic Test Evaluation with Software SERION *evaluate*

After input of the 4 parameters and the reference value of the standard serum, antigen activities are calculated online from processed and measured SERION ELISA *antigen* test runs by the evaluation software SERION *evaluate*.

If the optical density of the standard is out of the valid range, the following message will appear:

“Standard values out of ranges in following groups: Group 1-24.” or

“Standard values differ more than 20% in following groups: Group 1-24.”

In these cases the test run is invalid and should be repeated.

Parameters and reference value need to be changed only if there is a change of lot (evaluation table shows parameters and reference values). Correct input of the lot specific data can be checked on the basis of the standard serum activity (in IU/ml or U/ml assigned to the standard serum. The calculated mean value of the units has to correspond to the unit value indicated on the lot specific certificate. There is an automatic correction of the measured values. In the standard version the printout displays the following:

Sample code OD-value U/ml Evaluation

8.4 Limits of Quantification

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

8.5 Borderline Range

The borderline range of the SERION ELISA *antigen* Candida test is specified on the quality control certificate and indicates the range for borderline test results. Values obtained which fall below this range indicate a negative test result; values above the borderline range are interpreted as positive

for the antigen. In cases where the results are within the borderline range, a definitive interpretation of the result is not possible. In these cases, the test should be repeated.

9 Safety Measures

9.1 Statements of Warning

The SERION ELISA *antigen* is only designed for qualified personnel who are familiar with good laboratory practice.

All kit reagents and human samples should be handled carefully, using established good laboratory practice.

- This kit contains human blood components. Although all control- and cut-off-sera have been tested and found negative for HBs-Ag-, HCV- and HIV-antibodies, they should be considered potentially infectious.
- Do not pipette by mouth.
- Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
- Wear disposable gloves, laboratory coat and safety glasses while handling kit reagents or specimens. Wash hands thoroughly afterwards.
- Samples and other potentially infectious material should be decontaminated after the test run.
- Reagents should be stored safely and be unaccessible to unauthorized access e.g. children.

9.2 Disposal

Please observe the relevant statutory requirements!

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