

SERION ELISA *classic*

Candida albicans IgG/IgM/IgA

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SERION ELISA *classic* Candida albicans

Enzyme Immunoassay for detection of human antibodies (IgG/IgM/IgA)

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

IgG-Kit (quantitative)	order number:	ESR117G
IgM-Kit (quantitative)	order number:	ESR117M
IgA-Kit (quantitative)	order number:	ESR117A

Tests evaluated: Immunomat *TWIN*system / *BASE*plus, Dade Behring BEP ® III / BEP ® 2000, DSX, manually

1 INTENDED USE

SERION ELISA *classic* Candida albicans IgG/IgM/IgA are quantitative and qualitative tests for detection of human antibodies in serum or plasma directed against Candida albicans. The complex antigen that consists of cytoplasmic and a reduced amount of cell wall components enables the detection of anti-Candida antibodies in superficial and invasive mycoses . For sale in the U.S. for Research Use Only. Not for use in diagnostic procedures.

2 BACKGROUND

Candida albicans is a ubiquitous yeast that belongs to the family of yeastlike fungi (see table 1).

Table 1: Fungi of medical relevance

Fungi of medical relevance	
1. yeastlike fungi	Candida ssp. Torulopsis Cryptococcus
2. mold	Aspergillus ssp. Mucor ssp. Rhizopus ssp.
3. dermatophytes	
4. dimorphic 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 invasive mycosis.

Candida yeastlike fungi produce and excrete multiple enzymes that enable this organism to penetrate blood vessels and mucosa barriers. Subsequent to infestation of the mucosa, invasive mycosis spreads out from the gastro intestinal tract.

The attachment of yeastlike fungi to different mucosa of ephithelia is caused by adherent proteins and further structures of the outer cytomembrane. The following scheme shows (hypothetical) steps that may cause disseminated infections in severe cases. An exact discrimination of the stages is hardly possible (figure 1).

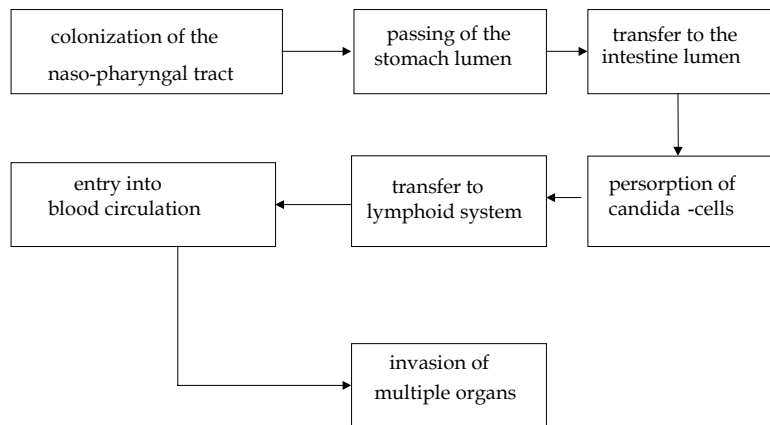


Figure 1: progression of candida-infections

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

In general human *Candida ssp.* infection is passed on by smear contamination. The main entrance is the naso-pharyngeal tract. Fungistatic malfunction of the skin surface caused by fatty acids, acid pH-value and antagonistic flora, may lead to superficial candidiasis.

3 SERION ELISA *classic* - TEST PRINCIPLE

Microtiter wells are coated with **antigens**. This constitutes the **solid phase**. Sample is added to the wells and any antibodies specific for the antigen present will bind to the solid phase. After removal of unbound material, anti-human **IgG or IgA or IgM conjugated** to an enzyme (**alkaline phosphatase**) is allowed to react with the immune complex. After removal of excess conjugate by washing, an appropriate **substrate (para-nitrophenylphosphate)** is added, with which the conjugated enzyme reacts producing a **colored derivative of the substrate**. The color intensity is proportional to the level of specific antibody bound and can be quantified photometrically.

4 COMPONENTS OF THE KIT

Test components	amount / volume
Break apart microtiter test strips each with 8 antigen coated single wells (altogether 96) [MTP] 1 frame the coating material is inactivated	12
Standard serum (ready-to-use) [STD] Human serum in phosphate buffer with protein; negative for anti-HIV-Ab, anti-HBs-Ag (Hepatitis B-Virus-surface antigen) and anti-HCV-Ab; preservative: < 0.1 % sodium azide colouring: Amaranth O	2 x 2 ml
Negative control serum (ready-to-use) [NEG] Human serum in phosphate buffer with protein; negative for anti-HIV, anti-HBs (Hepatitis B-Virus-surface antigen) and anti-HCV; preservative: < 0.1 % sodium azide colouring: Lissamin green V	2 ml
Anti-human-IgG-, IgA-, IgM-conjugate (ready-to-use) [APC] Anti-human-IgG, -IgA, -IgM from goat (polyclonal), conjugated to alkaline phosphatase, stabilized with protein stabilization solution preservative: 0.01 % methylisothiazolone, 0.01 % bromnitrodioxane	13 ml
Washing solution concentrate (sufficient for 1 litre) [WASH] Sodium chloride solution with Tween 20, 30 mM Tris preservative: < 0.1 % sodium azide	33.3 ml
Dilution buffer [DILB] Phosphate buffer with protein and Tween 20; preservative: < 0.1 % sodium azide 0.01 g/l Bromphenol blue sodium salt	2 x 50 ml
Stopping solution [STOP] 1.2 N sodium hydroxide	15 ml
Substrate (ready-to-use) [pNPP] Para-nitrophenylphosphate, solvent free buffer preservative: < 0.1 % sodium azide (Substrate in unopened bottle may have a slightly yellow coloring. This does not reduce the quality of the product!)	13 ml
Quality control certificate with standard curve and evaluation table [INFO] (quantification of antibodies in IU/ml or U/ml)	1

5 MATERIAL REQUIRED BUT NOT SUPPLIED

- common laboratory equipment
- for the IgM-ELISA: SERION Rf-Absorbent (Order no. Z200/20ml, Z4/4 ml)
- photometer for microtiter plates with filter, wavelength 405 nm, recommended reference wavelength 620 nm - 690 nm (e.g. 650 nm)
- incubator 37°C
- moist chamber
- distilled water

6 STORAGE AND STABILITY

Reagent	Storage	Stability
microtiter strips (antigen)	unopened after opening at 2-8°C in closed aluminum bag with desiccant <i>Strips which are not used must be stored in the press-seal bag of aluminum compound foil under dry and airtight conditions!</i>	see expiry date on microtiter plate 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 <i>Avoid contamination (sterile tips!)</i>	until expiry date 28 months from date of production
dilution buffer	after opening at 2-8°C <i>Discard cloudy solutions!</i> unopened	24 months until expiry date; 36 months from

		date of production
washing solution	concentrate after opening at 2-8°C working dilution at 2-8°C working dilution at room temperature <i>Bottles used for the working dilution should be cleaned regularly, discard cloudy solutions.</i>	until expiry date 2 weeks 1 week
substrate	ready-to-use solution at 2-8°C, protected from light! <i>Avoid contamination (sterile tips!) Discard when solution turns yellow (extinction against distilled water > 0.25).</i>	until expiry date 24 months from date of production
stopping solution	after opening at room temperature	until expiry date

7 TEST PROCEDURE SERION ELISA *classic*

7.1 Evidence of deterioration

Only use SERION ELISA *classic* reagents for test procedure, since all reagents are matched. In particular standard and control sera are defined exclusively for the test kit to be used. Do not use them in other lots. Dilution buffer, washing solution and substrate solution can be used for all SERION ELISA *classic* kits irrespective of the lot and the test.

There are three different conjugate concentrations for each immunoglobulin class: LOW, MEDIUM, HIGH

The classification is written on each label as follows:

e.g. IgG + lowly concentrated IgG conjugate
 IgG ++ medium concentrated IgG conjugate
 IgG +++ highly concentrated IgG conjugate

In rare cases the use of special conjugate is necessary to guarantee consistent quality for our products. Special conjugates are produced in a separate lot and do not carry the “+” sign. Therefore, special conjugates are not exchangeable with other conjugates.

Please pay close attention to notifications on labels!

Unopened, all components of the SERION ELISA *classic* kits may be used up to the dates given on the labels, if stored at +2°C to +8°C. Complete stability and storage data are described under “6. Storage and Stability “.

Each reagent has been calibrated and optimized for the test. Dilution or alteration of these reagents may result in a loss of sensitivity.

Avoid exposure of reagents to strong light during storage and incubation. Reagents must be tightly closed to avoid evaporation and contamination with microorganisms since incorrect test results could occur due to interference from proteolytic enzymes.

To open the press-seal bag please cut off the top of the marked side, only. Do not use the strips if the aluminum bag is damaged or if the press-seal bag with remaining strips and desiccant was not properly reclosed.

Bring all reagents to room temperature before testing.

Use aseptic techniques for removing aliquots from the reagent tubes to avoid contamination. To avoid false positive results ensure not to contact or sprinkle the top-walls of wells while pipetting conjugate. Take care not to mix the caps of the bottles and/or vials.

Reproducibility is dependent on thorough mixing of the reagents. Shake the flasks containing control sera before use and also all samples after dilution (e.g. by using 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 filling samples/control sera, conjugate or substrate may result in different “pre incubation” times, which may influence the precision and reproducibility of the results.

Optimum results can only be achieved if SERION ELISA *classic* instructions are followed strictly.

The test is not valid, if the lot-specific validation criteria on the quality control certificate are not fulfilled.

Inadequate washing will affect the test results:

The washing procedure should be carried out carefully. If the washing procedure is carried out automatically follow the instruction manual of the respective washer. Flat bottom wells are used for SERION ELISA *classic*. All 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 by tapping the inverted microtest plate on a paper towel. Avoid foam! Do not scratch coated wells during washing and aspiration. If using an automated washer, ensure it is operating correctly.

7.2 Sample preparation and storage

Lipaemic, hemolytic or icteric samples should only be tested with reservations although in our testing no negative influence has been found. Obviously contaminated samples (serum or plasma) should not be tested due to the risk of wrong results.

Serum or plasma (EDTA, citrate, heparin) collected according to standard laboratory methods are suitable samples.

Samples must not be thermally inactivated.

7.2.1 Sample preparation

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

SERION ELISA *classic* Candida albicans IgG and IgA:

$V_1 + V_2 = 1+1000$	add	10 μ l	serum
	each to	1000 μ l	dilution buffer (1 + 100)
		20 μ l	of first dilution
	each to	200 μ l	dilution buffer (1 + 10)

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

SERION ELISA *classic* Candida albicans IgM:

Rheumatoid factors are **autoantibodies mainly of the IgM-class**, which preferably bind to IgG-immune-complexes. The presence of non-specific IgM-antibodies (rheumatoid factors) can lead to **false-positive** results in the IgM-assay. Furthermore, the possibility exists, that weak-binding pathogen-specific IgM-antibodies are displaced by stronger-binding IgG-antibodies. In this case, IgM-detection can lead to **false-negative** results. Therefore it is necessary to pretreat samples with rheumatoid factor-absorbens prior to IgM detection (SERION Rheumatoid Factor-Absorbent, Order-No. Z200 (20 ml/100 tests)).

Before running the test, rheumatoid factor-absorbent (V₁) must be diluted 1+4 in dilution buffer (V₂).

$$V_1 + V_2 = 1 + 4$$

add 200 µl Rf-absorbent

each to 800 µl dilution buffer

Samples (V₄) must be diluted in this Rf-dilution buffer (V₃)

$$V_4 + V_3 = 1+100$$

add 10 µl sample

each to 1000 µl Rf-dilution buffer

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

7.2.2 Sample storage

The stoppered samples can be stored in a refrigerator up to 7 days at 2-8°C. Extended storage is possible at ≤ -20°C.

Avoid repeated freezing and thawing of samples.

Diluted samples can be stored at 2-8°C for one week.

7.3 Preparation of kit reagents

7.3.1 Microtest strips

Microtest strips in frame are packed with desiccant in an aluminum bag. Take unrequired cavities out of the frame and put them back into the press-seal bag. Close press-seal bag carefully to ensure airtight conditions.

7.3.2 Control sera / standard sera

Control and standard sera are ready-to-use and must not be diluted any further. They can be used directly for the test run.

For each test run and for each test system - independent of the number of microtest strips to be used - control and standard sera must be included. The cut-off-control should be set up in duplicate. With the quantitative tests the standard serum should also be set up in duplicate.

Do not treat control sera with Rf-absorbent.

7.3.3 Anti-human-IgG-, IgM- or IgA-AP-conjugate (ready-to-use)

Conjugates with the same concentration and within the same immunoglobulin class are exchangeable (see description in 7.1). Avoid contamination of ready-to-use conjugates (please pour sufficient for test into a secondary container to avoid repeatedly pipetting from the original bottle).

7.3.4 Washing solution

Dilute washing buffer concentrate (V_1) 1:30 with aqua dest. to a final volume of V_2 .

Example:

buffer concentrate (V_1)	final volume (V_2)
33.3 ml	1000 ml
1 ml	30 ml

7.3.5 Dilution buffer for samples (ready-to-use)

7.3.6 Substrate (ready-to-use)

For pipetting substrate solution use sterile tips only!

7.3.7 Stopping solution (ready-to-use)

7.4 Overview - test procedure

Candida albicans **IgG/IgM/IgA quantitative**

in case of IgM-detection absorption of rheumatoid factor!

sample dilution¹

IgM: 1 + 100

IgG/IgA: 1 + 1000

Pipette diluted samples and ready-to-use control sera /
standard sera into the microtest wells (100 µl)

↓

INCUBATION 60 min./37°C
moist chamber

↓

WASH (4 x 300µl [DIL] [WASH])²

↓

Pipette conjugate solution [APC] (100 µl)

↓

INCUBATION 30 min./37°C
moist chamber

↓

WASH (4 x 300µl [DIL] [WASH])²

↓

Pipette substrate solution [pNPP] (100 µl)

↓

INCUBATION 30 min./37°C
moist chamber

↓

Pipette stopping solution [STOP] (100 µl)

↓

READ EXTINCTION AT 405 nm

¹special dilution buffer for the following test kits: Borrelia IgG & IgM, EBV EA IgG, Parvovirus B19 IgM

²for manual use: at the end of the procedure tap plate on paper towel

7.5 Test procedure

1. Place the required number of cavities in the frame and prepare a protocol sheet.
2. Add each **100 µl of diluted sample or ready-to-use controls** into the appropriate wells of microtest strips. Spare one well for substrate blank, e.g.:

IgG/IgM/IgA quantitative	
well A1	substrate blank
well B1	negative control
well C1	standard serum
well D1	standard serum
well E1	sample 1....

3. **Sample incubation for 60 minutes (+/- 5 min) at 37°C (+/- 1°C) in moist chamber**
4. After incubation **wash** all wells with washing solution (by automated washer or manually):
 - aspirate or shake out the incubation solution
 - fill each well with 300 µl washing solution
 - aspirate or shake out the washing buffer
 - repeat the washing procedure 3 times (altogether 4 times!)
 - dry by tapping the microtest plate on a paper towel
5. **Addition of conjugate**
Add 100 µl of IgG-/IgM-/IgA-conjugate (ready-to-use) to the appropriate well (except substrate blank)
6. **Conjugate incubation for 30 minutes (+/- 1 min) * at 37°C (+/- 1°C) in moist chamber.**
7. After incubation **wash** all wells with washing solution (see above)
8. **Addition of substrate**
Add 100 µl substrate solution (ready-to-use) to each well (including well for substrate blank!)

9. **Substrate incubation for 30 minutes (+/- 1 min) * at 37°C (+/- 1°C) in moist chamber.**

10. **Stopping of the reaction**

Add 100 µl stopping solution to each well, shake microtest plate gently to mix.

11. **Read optical density**

Read OD within 60 minutes at 405 nm against substrate blank, reference wave length between 620 nm and 690 nm (e.g. 650 nm).

8 TEST EVALUATION SERION ELISA *classic* *Candida albicans* IgG/IgM/IgA

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 antibody concentrations with the SERION ELISA *classic* 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:

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

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.

* Please note, that under special working-conditions internal laboratory adaptations of the incubation times could be necessary.

For evaluation of antibody 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 producer. Within this range a correct quantification of antibody concentration is ensured. Since the standard serum is not necessarily a positive control, the value of the standard serum may be borderline or negative in some ELISA tests.

8.2 Criteria of validity

- the substrate blank must be $OD < 0.25$
- the negative control must be negative
- quantitative ELISA: the mean OD-value of the standard serum must be within the validity range, which is given on the lot specific quality control certificate of the kit (after subtraction of the substrate blank!)
- qualitative ELISA: the mean OD-value of the positive control must be within the validity range, which is given on the lot specific quality control certificate of the kit (after subtraction of the substrate blank!)
- the variation of OD-values 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 *classic* Candida albicans IgG/IgM/IgA (quantitative)

8.3.1 Non-automated evaluation

For the test evaluation a standard curve and an evaluation table are included in the test kit so that the obtained OD-values may be assigned to the corresponding antibody activity. The reference value and the validity range of the standard serum is given on the evaluation table (quality control certificate).

The blank (A1) must be subtracted from all OD-values prior to the evaluation.

Method 1: Qualitative Evaluation

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

$OD = 0.502 \times MW(STD)$ with upper cut-off

$OD = 0.352 \times MW(STD)$ with lower cut-off

If the measured mean absorbance value of the standard serum is 0.64, the range of the cut-off is in between 0.225-0.321.

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

So called *interassay variations* (day to day deviations and laboratory to laboratory deviations) are compensated 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 standard serum / mean value extinction standard serum.
3. All measured values of samples are multiplied by "F".
4. Antibody 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 SERION *easy base* 4PL-Software/SERION *evaluate*-Software

After input of the 4 parameters and the reference value of the standard serum, antibody activities are calculated online. If the optical density of the standard is out of the valid range, the following message will appear:

SERION *easy base* 4PL-Software:

"Standards are not in tolerance range" and/or "Distance between standards is greater than 20 %."

SERION *evaluate*-Software:

“Standard values out of ranges in following groups: Group 1-24. Standard value 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 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
IU/ml or U/ml
evaluation

9 STATEMENTS OF WARNING

9.1 Statements of warning

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

All kit reagents and human specimens 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.
- Stopping solution: corrosive (C); causes acid burn (R34)
use safety glasses, gloves and laboratory coat while handling!

9.2 Disposal

Please observe the relevant statutory requirements!

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