Candida albicans ELISA IgG/ IgM Testkit
IgA Set

Order No.: EC111.00 (IgG/IgM Testkit)
EC111.08 (IgA Set)

Color Coding: dark brown

FOR IN VITRO DIAGNOSIS ONLY
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1. **Intended Use**

The Candida albicans ELISA IgG/IgM/IgA is intended for semiquantitative and qualitative detection of IgG, IgM and IgA antibodies against Candida albicans in human serum. The test is to be used when clinical suspicion of invasive or systemic Candida-mycosis is present. Due to high prevalence the Candida albicans ELISA IgG is specially adjusted to rather detect early infections.

2. **Diagnostic Relevance**

The genus Candida belongs to the Blastomyces and is found as harmless commensale on the mucous (mouth, intestinal flora) in about 40% of the healthy population. Transmission mainly happens by smear contamination from human to human. Often an unstable immune status, especially a cellular immune response deficiency, is cause of a Candida infection.

A surface candidosis develops by increasing growth and attachment of Candida species to the epithelcells. If the microorganism succeeds in conquering the mucous barrier and to pass over to the lymphatic system and blood circulation, the result is the colonisation of the inner organs and the development of a systemic, invasive Candidosis. (1)

<table>
<thead>
<tr>
<th>Candida Infections</th>
<th>Surface</th>
<th>Invasive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Localization</td>
<td>skin, mucous</td>
<td>generalized</td>
</tr>
<tr>
<td>Immune status</td>
<td>+</td>
<td>+, +/-, -</td>
</tr>
<tr>
<td>Risk factors</td>
<td>pregnancy, diabetes, AIDS, disturbances of skin properties</td>
<td>chemotherapy, iatrogenic immunosuppression, central vein catheter, radiotherapy, burn, surgical intervention</td>
</tr>
<tr>
<td>Course</td>
<td>mainly harmless</td>
<td>often life-threatening</td>
</tr>
<tr>
<td>Possibility of therapy</td>
<td>good</td>
<td>depends on stage</td>
</tr>
</tbody>
</table>

The progress of an invasive candidosis is very uncharacteristic, this makes an early recognition of an infection based upon symptoms and signs very difficult. The detection of Candida-antigen and / or specific Candida-antibodies in human serum gains in importance. It has to be taken into account that a temporary colonisation with Candida can also induce an antibody-response. On the opposite only low-grade titer changes may appear with a systemic Candida infection in immunesuppresive patients. (1,2)

Generally it has to be remarked that the different identification methods are based on different test principles, what makes a direct comparison of the tests difficult. The specificity of the antibodies detected mainly depends on the antigen preparation used (e.g. HAT, ELISA, IFA). Mainly antibodies of the IgM type are detected with the HAT because of their marked agglutination pattern. As titer courses of the immunoglobulin classes cannot be followed up separately it may come to a simulation of stagnating titers while IgM titers fall and IgG titers rise synchronously. A combination of HAT and ELISA enables the ELISA to show titer changes while the HAT-titer stagnates. (3,4)

As non-treated invasive infections may proceed fatal, it is recommendable to check risk patients in a one week interval to Candida antibodies. At an acute suspicion of an invasive Candidosis, additional serum samples should be tested. For optimal monitoring and therapy control a combination of further test methods should also be used, as no test method on itself may offer a comprehensive Candida diagnosis. Often immune suppressed patients and children show lower antibody concentrations, too. Titer movements below the limit values can in these cases already be remarked as a notice of a Candidosis. The procedure of additional test methods is absolutely to recommend in such cases. Due to the high prevalence, the Virotech ELISA IgG has been adjusted using specific sera with proved Candida infection, to rather detect fresh infections in IgG. (1,3)
3. **Test Principle**

The antibody searched for in the human serum forms an immune complex with the antigen coated on the microtiter-plate. Unbound immunoglobulins are removed by washing processes. The enzyme conjugate attaches to this complex. Unbound conjugate is again removed by washing processes. After adding the substrate solution (TMB), a blue dye is produced by the bound enzyme (peroxidase). The color changes to yellow when the stopping solution is added.

4. **Package Contents**

4.1 **IgG/IgM Testkit**

1. **1 Microtiter-Plate** consisting of 96 with antigen coated, breakable single wells, lyophilised
2. **PBS-Dilution Buffer (blue, ready to use) 2x50ml**, pH 7,2, with preservative and Tween 20
3. **PBS-Washing Solution (20x concentrated) 50ml**, pH 7,2, with preservative and Tween 20
4. **IgG negative Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
5. **IgG cut-off Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
6. **IgG positive Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
7. **IgM negative Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
8. **IgM cut-off Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
9. **IgM positive Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
10. **IgG-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with protein-stabilizer and preservative in Tris-Buffer, ready to use
11. **IgM-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase with FCS and preservative in Tris-Buffer, ready to use
12. **Tetramethylbenzidine substrate solution (3,3',5,5'-TMB), 11ml**, ready to use
13. **Citrate-Stopping Solution, 6ml**, contains an acid mixture

4.2 **IgA-Set**

1. **IgA negative Control, 1300µl**, human serum with protein stabilizer and preservative, ready to use
2. **IgA cut-off Control, 1300µl**, human serum with protein stabilizer and preservative, ready to use
3. **IgA positive Control, 1300µl**, human serum with protein stabilizer and preservative, ready to use
4. **IgA Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase with FCS and preservative in Tris-Buffer, ready to use

5. **Storage and Shelflife of the Testkit and the ready to use reagents**

Store the testkit at 2-8°C. The shelf life of all components is shown on each respective label; for the kit shelf life please see Quality Control Certificate.

1. Microtiter strips/single wells are to be resealed in package after taking out single wells and stored with desiccant at 2-8°C. Reagents should immediately be returned to storage at 2-8°C after usage.
2. The ready to use conjugate and the TMB-substrate solution are sensitive to light and have to be stored in dark. Should there be a color reaction of the substrate dilution due to incidence of light, it is not useable anymore.
3. Take out only the amount of ready to use conjugate or TMB needed for the test insertion. Additional conjugate or TMB taken out may not be returned but must be dismissed.

<table>
<thead>
<tr>
<th>Material</th>
<th>Status</th>
<th>Storage</th>
<th>Shelflife</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Samples</td>
<td>Diluted</td>
<td>+2 to +8°C</td>
<td>max. 6h</td>
</tr>
<tr>
<td>Controls</td>
<td>Undiluted</td>
<td>+2 to +8°C</td>
<td>1 week</td>
</tr>
<tr>
<td>Microtitreplate</td>
<td>After Opening</td>
<td>+2 to +8°C</td>
<td>3 months</td>
</tr>
<tr>
<td>Rheumatoid factor -</td>
<td>Undiluted, After Opening</td>
<td>+2 to +8°C</td>
<td>3 months</td>
</tr>
<tr>
<td>Absorbent</td>
<td>Diluted</td>
<td>+2 to +8°C</td>
<td>1 week</td>
</tr>
</tbody>
</table>
6. Precautions and Warnings

1. Only sera which have been tested and found to be negative for HIV-1 antibodies, HIV-2 antibodies, HCV antibodies and Hepatitis-B surface-antigen are used as control sera. Nevertheless, samples, diluted samples, controls, conjugates and microtiter strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.

2. Those components that contain preservatives, the Citrate Stopping Solution and the TMB have an irritating effect to skin, eyes and mucus. If body parts are contacted, immediately wash them under flowing water and possibly consult a doctor.

3. The disposal of the used materials has to be done according to the country-specific guidelines.

7. Material required but not supplied

1. Aqua dest./demin.
2. Eight-channel pipette 50µl, 100µl
3. Micropipettes: 10µl, 100µl, 1000µl
4. Test tubes
5. Paper towels or absorbent paper
6. Cover for ELISA-plates
7. Disposal box for infectious material
8. ELISA handwasher or automated EIA plate washing device
9. ELISA plate spectrophotometer, wavelength = 450nm, reference length = 620nm (Reference Wavelength 620-690nm)
10. Incubator

8. Test Procedure

Working exactly referring to the Virotech Diagnostics user manual is the prerequisite for obtaining correct results.

8.1 Examination Material

Either serum or plasma can be used as test material, even if only serum is mentioned in the instructions. Any type of anticoagulant can be used for plasma.

Always prepare patient-dilution freshly.

For a longer storage the sera must be frozen. Repeated defrosting should be avoided.

1. Only fresh non-inactivated sera should be used.
2. Hyperlipaemic, haemolytic, microbially contaminated and turbid sera should not to be used (false positive/negative results).

8.2 Preparation of Reagents

The Virotech Diagnostics System Diagnostica offers a high degree of flexibility regarding the possibility to use the dilution buffer, washing solution, TMB, citrate stopping solution as well as the conjugate for all parameters and for all different lots. The ready to use controls (positive control, negative control, cut-off control) are parameter specific and only to use with the plate lot indicated in the Quality Control Certificate.

1. Set incubator to 37°C and check proper temperature setting before start of incubation.
2. Bring all reagents to room temperature before opening package of microtiter strips.
3. Shake all liquid components well before use.
4. Make up the washing solution concentrate to 1 L with distilled or demineralised water. If crystals have formed in the concentrate, please bring the concentrate to room temperature before use and shake well before use.
5. High IgG-titer or rheumatoid factors may disturb the specific detection of IgM-antibodies and may lead to false positive resp. false negative results. **For a correct IgM-determination it is therefore necessary to pre-treat the sera with RF-SorboTech** (VIROTECH adsorbent). For IgM-controls a pre-absorbent treatment is not necessary.
8.3 Virotech ELISA Test Procedure

1. For each test run, pipette 100 µl each of ready to use dilution buffer (blank), IgG- and IgM-positive, negative and cut-off controls as well as diluted patient sera. We propose a double insertion (blank, controls and patient sera); for cut-off control a double insertion is absolutely necessary.

Working dilution of patient sera:

<table>
<thead>
<tr>
<th>Detection of</th>
<th>Patient serum</th>
<th>RF-SorboTech</th>
<th>Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG and IgA</td>
<td>5 µl</td>
<td>-</td>
<td>500 µl pre-dilution (1:101)</td>
</tr>
<tr>
<td></td>
<td>30 µl of the 1:101 pre-dilution</td>
<td>-</td>
<td>270 µl final dilution (1:1010)</td>
</tr>
<tr>
<td>IgM</td>
<td>5 µl</td>
<td>45 µl</td>
<td>450 µl pre-dilution (1:100)</td>
</tr>
<tr>
<td></td>
<td>30 µl of the 1:100 pre-dilution</td>
<td>-</td>
<td>270 µl final dilution (1:1000)</td>
</tr>
</tbody>
</table>

(Please remark the pre-absorption for IgM-diagnostic!)

2. After pipetting start incubation for 30 min. at 37°C (with cover).
3. End incubation period by washing microtiter strips 4 times with 350 – 400 µl washing solution per well. Do not leave any washing solution in the wells. Remove residues on a cellulose pad.
4. Pipette 100 µl of ready to use conjugate into each well.
5. Incubation of conjugates: 30 min. at 37°C (with cover).
6. Stop conjugate incubation by washing 4 times (pls. refer to point 3 above).
7. Pipette 100 µl of ready to use TMB into each well.
8. Incubation of substrate solution: 30 min. at 37°C (with cover, keep in dark).
9. Stopping of substrate reaction: pipette 50 µl of citrate stopping solution into each well. Shake plate carefully and thoroughly until liquid is completely mixed and a homogeneous yellow color is visible.
10. Measure extinction (OD) at 450/620nm (Reference Wavelength 620-690nm). Set your photometer in such a way that the blank value is deducted from all other extinctions. Extinctions should be measured within 1 hour after adding the stopping solution!

Pls. refer to last page for Test Procedure Scheme

8.4 Usage of ELISA processors

All Virotech Diagnostics ELISAs can be used on ELISA processors. The user is bound to proceed a validation of the devices (processors) on a regular basis.

Virotech Diagnostics recommends the following procedure:

1. Virotech Diagnostics recommends to proceed the validation of device referring to the instructions of the device manufacturer during the implementation of the ELISA processor respectively after bigger reparations.
2. It is recommended to check the ELISA-processor with the Validationkit (EC250.00) afterwards. A regular check using the Validationkit shall be proceeded minimum once a quarter to test the accuracy of the processor.
3. The release criteria of the Quality Control Certificate of the product must be fulfilled for each testrun.

With this procedure, your ELISA processor will function properly and this will support quality assurance in your laboratory.

9. Test Evaluation

The ready to use controls serve for a semiquantitative determination of specific IgG- and IgM-antibodies. Their concentration can be expressed in Virotech units = VE. Fluctuations resulting from the test procedure can be balanced with this calculation method and a high reproducibility is achieved in this way. Use the means of the OD values for calculation of the VE.
9.1 Test function control
   a) OD-values
      The OD of the blank should be < 0.15.
      The OD-values of the negative controls should be lower than the OD-values mentioned in the Quality Control Certificate. The OD-values of the positive controls as well as of the cut-off controls should be above the OD-values mentioned in the Quality Control Certificate.

   b) Virotech Units (VE)
      The Virotech Units (VE) of the cut-off controls are defined as 10. The calculated VE of the positive controls should be within the ranges mentioned in the Quality Control Certificate.
      If those requirements (OD-values, VE) are not fulfilled, the test has to be repeated.

9.2 Calculation of the Virotech Units (VE)
   The extinction of the blank value (450/620nm) has to be subtracted from all other extinctions.

\[
\begin{align*}
VE \text{ (positive control)} &= \frac{\text{OD (positive control)}}{\text{OD (cut-off control)}} \times 10 \\
VE \text{ (patient serum)} &= \frac{\text{OD (patient serum)}}{\text{OD (cut-off control)}} \times 10
\end{align*}
\]

9.3 Interpretation Scheme IgG, IgM and IgA

<table>
<thead>
<tr>
<th>Result (VE)</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 9,0</td>
<td>negative</td>
</tr>
<tr>
<td>9,0 - 11,0</td>
<td>borderline</td>
</tr>
<tr>
<td>&gt; 11,0</td>
<td>positive</td>
</tr>
</tbody>
</table>

1. If the measured values are above the defined borderline range, they are considered to be positive.
2. If the measured VE is within the borderline range, no significant high antibody concentration is present, the samples are considered to be borderline. For the secure detection of an infection it is necessary to determine the antibody concentration of two serum samples. One sample shall be taken directly at the beginning of the infection and a second sample 5 – 10 days later (convalescent serum). The antibody concentration of both samples has to be tested in parallel, that means in one test run. A correct diagnosis based on the evaluation of a single serum sample is not possible.
3. If the measured values are below the defined borderline range, no measurable antigen specific antibodies are present in the samples. The samples are considered to be negative.
4. A positive IgG result indicates either a past infection or a recent infection.
   A positive IgM result indicates an acute infection and a positive IgA result indicates a relatively acute infection, as IgA can persist for months.
   A negative result indicates, that the patient is/was not infected.
9.4 Interpretation Scheme

<table>
<thead>
<tr>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>no notice of an invasive candidosis</td>
</tr>
<tr>
<td>+/(borderline)</td>
<td>-</td>
<td>-</td>
<td>notice of passed infection</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>notice of acute infection</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

9.5 Limits of the Test

1. The interpretation of serological results shall always include the clinical picture, epidemiological data and all further available laboratory results.
2. Cross-reactivities may be especially expected with sera which are positive for other Candida species, Mycoplasma species or Penicillium marneffei.
3. Due to the high prevalence of Candida often high antibody concentrations are found, especially for IgG. Isolated high IgG titers do therefore not lead to the presence of an invasive Candidosis.

To be able to treat a possibly life-threatening Candidosis early, it is recommendable to test the serum samples of risk patients in an interval of one week. At acute suspicion of an invasive Candidosis additional serum samples should be tested.

It has to be considered that significant titer courses below the limit values, especially at immunosuppressed patients and children can be a notice of an invasive infection. The interpretation of the ELISA results should always be effected in combination with additional test methods (HAT, culture), further laboratory diagnostic parameters and the clinical picture. A correct diagnosis due to the assessment of a single serum sample is impossible.

10. Performance Data

10.1 Diagnostical sensitivity

For the detection of the diagnostical sensitivity 34 sera were tested. These sera are from routine tests by the University hospital Heidelberg with positive blood culture result as well as one serum with histological assessment. The comparison was made to the blood culture result respectively the histological assessment.

The minimum criterion for a notice to an invasive Candidosis in the Virotech ELISA is a positive result of the respective serum in IgA- or IgM-Candida-ELISA.

<table>
<thead>
<tr>
<th>Seracollective (n=34)</th>
<th>IgM/IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>9</td>
</tr>
<tr>
<td>Borderline</td>
<td>2</td>
</tr>
<tr>
<td>Positive</td>
<td>23</td>
</tr>
</tbody>
</table>

The diagnostical sensitivity is 71,9%.

Borderline results have not been considered into calculation.
10.2 Specificity
For the determination of the specificity 80 blood donor sera were tested in IgM and IgA.

<table>
<thead>
<tr>
<th>Seracollective (n=80)</th>
<th>IgM</th>
<th>IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>75</td>
<td>80</td>
</tr>
<tr>
<td>Borderline</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Positive</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

The specificity for IgA is >99,8% and for IgM is 96,6%.
Borderline results have not been considered.

10.3 Cross Reactivity
For checking the cross reactivity 82 routine sera from the university hospital Heidelberg, which are positive against 12 different pathogens (e.g. Aspergillus species, Penicillum marneffei) were examined.
The thus calculated specificity is 87,3%.
Cross-reactivities may be especially expected with sera which are positive for other Candida species, Mycoplasma species or Penicillum marneffei.

10.4 Prevalence (Expected Values)
The following table shows the results of the examination of blood bank sera in IgG (n=80), IgA (n=80) and IgM (n=80)

<table>
<thead>
<tr>
<th></th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>65</td>
<td>80</td>
<td>75</td>
</tr>
<tr>
<td>Borderline</td>
<td>8</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Positive</td>
<td>7</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

10.5 Intra-assay-Coefficient of Variation (Repeatability)
In one assay, strips of different plates of one batch have been tested with the same serum sample. The obtained coefficient of variation is < 9% (at an average OD-value of 0.67).

10.6 Inter-assay-Coefficient of Variation (Reproducibility)
Three sera were tested in 10 independent test runs by different persons.

<table>
<thead>
<tr>
<th>Candida albicans ELISA IgG</th>
<th>Average Value VE</th>
<th>Coefficient of Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>4,97</td>
<td>13,87%</td>
</tr>
<tr>
<td>Borderline</td>
<td>9,47</td>
<td>7,83%</td>
</tr>
<tr>
<td>Positive</td>
<td>18,52</td>
<td>7,08%</td>
</tr>
</tbody>
</table>

11. Literature
1. T. Steinmetz; Candidamykosen in der Intensivmedizin; Mykosen Nr. 1, 1996, Seite 1-20
2. J. Krämer; Entwicklung neuer serologischer Testverfahren zur Diagnostik der invasiven Candidose auf der Basis von Markerantigenen; 1991; Doktorarbeit
### Test Procedure Scheme

#### Preparation of Patient Samples and Washing Solution

▼ **Washing Solution:** Fill up concentrate to 1 liter with aqua dest./demin.

▼ **IgG/IgA-Samples – Dilution 1:1001**

- e.g.:
  - 5 µl serum/plasma + 500 µl Dilution Buffer (1:101)
  - 30µl of the 1:101 pre-dilution + 270 µl Dilution buffer (Serum Dilution Buffer is ready to use)

▼ **IgM-Samples – Dilution 1:1000**

- Rheumafactor-absorption with RF-SorboTech
  - e.g.:
    - 5 µl serum/plasma + 450 µl Dilution Buffer + 1 drop RF-SorboTech, incubate for 15 min. at room temperature.
    - 30µl of the 1:100 pre-dilution + 270 µl Dilution buffer

#### Testprocedure

<table>
<thead>
<tr>
<th>Samples Incubation</th>
<th>30 minutes at 37°C</th>
<th>100 µl Patient Samples</th>
<th>blank value (Dilution Buffer) and controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wash 4times</td>
<td></td>
<td></td>
<td>400 µl Washing Solution</td>
</tr>
<tr>
<td>Conjugate Incubation</td>
<td>30 minutes at 37°C</td>
<td>100 µl Conjugate</td>
<td>IgG, IgM, IgA</td>
</tr>
<tr>
<td>Wash 4times</td>
<td></td>
<td></td>
<td>400 µl Washing Solution</td>
</tr>
<tr>
<td>Substrate Incubation</td>
<td>30 minutes at 37°C</td>
<td>100 µl Substrate</td>
<td></td>
</tr>
<tr>
<td>Stopping</td>
<td></td>
<td></td>
<td>50 µl Stopping Solution</td>
</tr>
<tr>
<td>Measure Extinctions</td>
<td></td>
<td></td>
<td>Photometer at 450/620nm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Reference Wavelength 620-690nm)</td>
</tr>
</tbody>
</table>