THE PLATELIA™ CANDIDA AG IS AN IMMUNOENZYMATIC SANDWICH MICROPLATE ASSAY FOR THE DETECTION OF CANDIDA MANNAN ANTIGEN IN SERUM
1- INTENDED USE
The Platelia™ Candida Ag is an immunoenzymatic sandwich microplate assay for the detection of circulating Candida mannan antigen in serum samples.

2- INDICATIONS FOR USE
The Platelia™ Candida Ag (cat. # 62798) is a test which, when used in combination with the Platelia™ Candida Ab/Ac/Ak (cat. # 62799) allows an earlier diagnosis and improves the sensitivity of the diagnosis of invasive candidiasis as part of a complete diagnostic approach integrating clinical and mycological data and evaluation of intrinsic and iatrogenic risk factors. This combination is also an integral part of a clinical and laboratory patient monitoring system as an aid to treatment decisions.

3- SUMMARY AND EXPLANATION
Candida infections are the most frequent form of nosocomial fungal infections. Invasive forms represent the most serious infections with mortality rates ranging from 30 to 70% in immunodepressed subjects. The diagnosis of invasive Candida infections is difficult to establish due to the poor specificity of clinical symptoms and the low sensitivity of cultures, despite the considerable progress recently made in this field. The diagnosis of invasive candidiasis, leading to initiation of appropriate treatment, is usually based on a combination of clinical, mycological and risk-factor considerations. In this context, the diagnosis of systemic candidiasis must always comprise serological techniques as well as direct mycological methods. The detection of circulating antigens in the serum appears to improve the diagnosis of Candida infection, particularly in immunodepressed subjects. Mannan, one of several Candida antigens, is a polysaccharide non-covalently bound to the yeast cell-wall and represents more than 7% of the dry weight of C. albicans. This antigen appears to be one of the main markers of invasive candidiasis.

4- PRINCIPLE OF THE PROCEDURE
The Platelia™ Candida Ag is a one-stage immunoenzymatic sandwich microplate assay which allows quantitative or qualitative detection of the circulating mannan antigen in human serum. The assay uses the rat monoclonal antibody EBCA-1, which is directed against Candida α 1-5 oligomannosides, and has been characterized in previous studies. The monoclonal antibody is used to:
• coat the wells of the microplate and bind the mannan antigen
• detect the antigen bound to the sensitized microplate (conjugate reagent: peroxidase-linked monoclonal antibody).
Serum samples are heat-treated in the presence of EDTA in order to dissociate immune complexes and to precipitate serum proteins that could possibly interfere with the test.

The treated serum samples and conjugate are added to the wells coated with monoclonal antibody, and incubated. A monoclonal antibody - mannan - monoclonal antibody / peroxidase complex is formed in the presence of mannan antigen. The strips are washed to remove any unbound material. Next, the substrate solution is added, which will react with the complexes bound to the well to form a blue color reaction. The enzyme reaction is stopped by the addition of acid, which changes the blue color to yellow. The absorbance (optical density) of specimens, controls and range points is determined with a spectrophotometer set at 450 and 620 nm wavelength.

The test can be performed according to two modes:

- Quantitative mode: by establishing a calibration curve from 4 range points: 2.0, 1.0, 0.5 and 0.25 ng/mL. The results of test samples are expressed in ng of mannan per mL of serum.
- Qualitative mode: only the 0.25 ng/mL and 0.5 ng/mL range points are used; comparison between OD of test samples and OD of the 2 range points permits expression of results as negative, intermediate or positive sera.

5- **REAGENTS**

Platelia™ *Candida Ag*: product No. 62798 (96 Tests)

Store the kit at +2-8°C. Bring all reagents to room temperature (+18-25°C) before use. Return all reagents to +2-8°C immediately after use. Return unused strips/plates to pouch and reseal. Do not remove desiccant. Strips should be used within 5 weeks of opening and resealing the pouch. After dilution, Working Washing Solution can be kept for 14 days at +2-8°C. All other reagents are stable until expiration after opening. Reagents are supplied in sufficient quantity to perform 96 tests in a maximum of 6 batches.
<table>
<thead>
<tr>
<th>Component</th>
<th>Contents</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1 Microwell Strip Plate</td>
<td><strong>Microplate Strip Plate:</strong> 96 wells (12 strips of 8 wells each) coated with EBCA-1 anti-mannan monoclonal antibody</td>
<td>1 Plate / 12 x 8 Wells</td>
</tr>
<tr>
<td>R2 Concentrated Washing Solution (10x)</td>
<td><strong>Concentrated Washing Solution (10x):</strong> TRIS-NaCl buffer (pH 7.4), 1% Tween® 20, Preservative: 0.01% thimerosal</td>
<td>1 x 100 mL</td>
</tr>
<tr>
<td>R3 Negative Control Serum</td>
<td><strong>Negative Control Serum:</strong> Human control serum negative for mannan, used as negative control and diluent for the standard serum during the preparation of the 1.0, 0.5 and 0.25 ng/mL range points in the quantitative test and for preparation of the calibrator serum in the qualitative test (see &quot;10-Procedure&quot;) - Negative for anti-HIV-1, anti-HIV-2, anti-HCV antibodies and HBs antigen - Preservative: &lt; 0.1% sodium azide</td>
<td>1 x 10 mL</td>
</tr>
<tr>
<td>R4 Calibrator Serum</td>
<td><strong>Calibrator Serum:</strong> Human serum containing 2.0 ng/mL of mannan, used for preparation of the 1.0, 0.5 and 0.25 ng/mL range points in the quantitative test and for preparation of the calibrator serum in the qualitative test (see &quot;10-Procedure&quot;) - Negative for anti-HIV-1, anti-HIV-2, anti-HCV antibodies and HBs antigen - Preservative: &lt; 0.1% sodium azide</td>
<td>2 x 2 mL</td>
</tr>
<tr>
<td>R5 Positive Control Serum</td>
<td><strong>Positive Control Serum:</strong> Human serum containing between 0.5 and 1.5 ng of mannan - Negative for anti-HIV-1, anti-HIV-2, anti-HCV antibodies and HBs antigen - Preservative: &lt; 0.1% sodium azide</td>
<td>1 x 2 mL</td>
</tr>
<tr>
<td>R6 Conjugate</td>
<td><strong>Conjugate (ready to use):</strong> Anti-mannan monoclonal antibody / peroxidase labeled - Preservative: 0.01% thimerosal</td>
<td>1 x 8 mL</td>
</tr>
</tbody>
</table>
TMB (Tetramethylbenzidine) is a non-carcinogenic and non-mutagenic chromogen for peroxidase.

**6- WARNINGS FOR USERS**

1. For *in vitro* diagnostic use.
2. For professional use only.
3. Use of this test kit with samples other than human serum is not recommended.
4. The human material used in the preparation of the reagents has been tested and found to be negative for anti-HIV-1, anti-HIV-2, and anti-HCV antibodies, as well as HBs antigen. However, as no method can absolutely guarantee the absence of infectious agents, all reagents and all patient samples should be handled as though capable of transmitting infection. All tests should be conducted using the precautions recommended for blood borne pathogens.
5. Wear protective clothing and disposable gloves while handling the kit reagents and patient samples. Wash hands thoroughly after performing the test.
6. Do not pipette by mouth.
7. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
8. Avoid splashing samples or solutions containing them.
9. Biological spills not containing acid should be wiped thoroughly with an effective disinfectant. Disinfectants that can be used include (but are not limited to) a solution of 10% bleach (0.5% solution of sodium hypochlorite).

<table>
<thead>
<tr>
<th>Component</th>
<th>Contents</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>R7 Serum Treatment Solution</td>
<td>Serum Treatment Solution (Ready-to-use): - EDTA acid solution, without preservative</td>
<td>1 x 10.5 mL</td>
</tr>
<tr>
<td>R8 TMB Substrate Buffer</td>
<td>TMB Substrate Buffer (Ready-to-use): - Citric acid and sodium acetate solution pH 5.2 - 0.009% Hydrogen peroxide - 4% Dimethylsulfoxide (DMSO)</td>
<td>1 x 60 mL</td>
</tr>
<tr>
<td>R9 Chromogen: TMB Solution</td>
<td>Chromogen: TMB Solution (concentrated): - 90% Dimethylsulfoxide (DMSO) solution containing 0.6% tetramethylbenzidine (TMB)*</td>
<td>1 x 1 mL</td>
</tr>
<tr>
<td>R10 Stopping Solution</td>
<td>Stopping Solution (Ready-to-use): - 1.5 N Sulphuric acid (H₂SO₄)</td>
<td>1 x 12 mL</td>
</tr>
<tr>
<td>Plate sealers</td>
<td>Plate sealers: - Adhesive sheets for microplates</td>
<td>1 x 4 sheets</td>
</tr>
</tbody>
</table>

*Note: TMB (Tetramethylbenzidine) is a non-carcinogenic and non-mutagenic chromogen for peroxidase.*
hypochlorite), 70% ethanol, or 0.5% Wescodyne™. Spills containing acid should be wiped dry or neutralized with sodium bicarbonate and then cleaned with one of the chemical disinfectants. Materials used to wipe up spills should be disposed of as biohazardous waste.

**CAUTION:** Do not place solutions containing bleach in the autoclave.

10. Dispose of all specimens and materials used to perform the test as though they contain an infectious agent. Disposal should comply with all applicable waste disposal requirements.

11. Some reagents contain sodium azide as preservative. Sodium azide can form lead or copper azides in the laboratory plumbing. These azides are explosive. To avoid any accumulation of azides, rinse the plumbing abundantly with water when discarding solutions containing azide in the sink after their inactivation.

12. **CAUTION:**

   Sulfuric acid (H₂SO₄) 1.5 N and DMSO (dimethylsulfoxide) 90%
   R 36/38: Irritating to eyes and skin.
   S:2-26-30: Keep out of reach of children. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Never add water to this product.

13. Avoid contact of TMB Substrate Buffer, Chromogen: TMB Solution, and Stopping Solution with eyes, skin, and mucosae (risk of toxicity, irritation, and burns).

14. The Material Safety Data Sheet (MSDS) is available upon request.

**7- PRECAUTIONS FOR USERS**

1. FROZEN SERUM SAMPLES STORED IN UNKNOWN CONDITIONS MAY GIVE FALSE POSITIVE RESULTS DUE TO CONTAMINATION WITH FUNGUS AND/OR BACTERIA.

2. Do not use kit or any kit reagents after the stated expiration date.

3. With the exception of the Concentrated Washing Solution (R2) and the Stopping Solution (R10), do not mix reagents from other kits that have different lot numbers.

4. Bring all reagents to room temperature for at least 15 minutes before use.

5. Mix thoroughly while reconstituting reagents, taking care to avoid microbial contamination.

6. Do not conduct the test in the presence of reactive vapors (acids, alkalis, aldehydes) or dust, which could affect the enzymatic activity of the Conjugate.
7. Use clean, disposable polypropylene plastic containers to prepare the Substrate-Chromogen Reaction Solution. If glassware must be used, it should first be washed in 1N hydrochloric acid, rinsed with distilled water, and dried.

8. For manual pipetting of controls and specimens, use individual pipette tips to prevent carryover of samples.

9. To ensure adequate washing of the wells, comply with the recommended number of wash cycles and ensure that all wells are completely filled and then completely emptied. Washing should not be performed manually with a squeeze bottle.

10. Do not allow the microplate to dry between the end of the wash cycle and addition of reagents.

11. Do not use the same container for the conjugate and substrate solutions.

12. Do not allow Conjugate or Substrate-Chromogen Reaction Solutions to come into contact with metal or metallic ions.

13. Avoid exposing the Chromogen: TMB Solution or the Substrate-Chromogen Reaction Solution to strong light during storage or incubation. Do not allow the chromogen solutions to come into contact with an oxidizing agent.

14. Avoid contact of the Stopping Solution with any oxidizing agent. Do not allow the Stopping Solution to come into contact with metal or metallic ions.

15. Limit exposure of solutions (sera, Serum Treatment Solution, Conjugate) or open containers (plates, tubes, pipettes) to the air.

16. Do not pour any unused Conjugate back into the original container.

17. The Substrate-Chromogen Reaction Solution must be colorless. The appearance of a blue color after dilution indicates the reagent is contaminated and should not be used. Discard and prepare fresh reagent.

8- REAGENT PREPARATION AND STORAGE

Microwell Strip Plate (R1)
After opening the plate pouch, the microwell strips are stable for 5 weeks when stored at +2-8°C in their carefully closed original bag in the presence of the enclosed desiccant.

Washing Solution (R2)
Prepare Working Washing Solution as needed by adding one part Concentrated Washing Solution to 9 parts sterile deionized or distilled water. Prepare a sufficient amount of Working Washing Solution to complete the run (80 mL for one strip: 8 mL R2 + 72 mL distilled water).
The Working Washing Solution can be stored for 14 days at +2-8°C. After opening, the Concentrated Washing Solution stored at +2-25°C, in the absence of contamination, is stable until the expiration date indicated on the label.

**Substrate-Chromogen Reaction Solution (R8+R9)**
Prepared Substrate-Chromogen Reaction Solution by adding one part concentrated Chromogen: TMB Solution, R9, to 50 parts TMB Substrate Buffer, R8. Prepare 2 mL of Substrate-Chromogen Reaction Solution per strip: 40 µL of R9 + 2 mL of R8.
The solution is stable for 6 hours when stored in the dark at room temperature (+18-25°C). After opening, R8 and R9 reagents stored at +2-8°C, in the absence of contamination, are stable until the expiration date indicated on the label.

**Negative Control Serum (R3), Calibrator Serum (R4), Positive Control Serum (R5), Conjugate (R6), Serum Treatment Solution (R7) and Stopping Solution (R10)**
After opening, R3, R4, R5, R6, R7 and R10 reagents stored at +2-8°C, in the absence of contamination, are stable until the expiration date indicated on the label.

**9- SPECIMEN COLLECTION**
Collect blood samples according to standard laboratory procedures. The test is performed on serum. Serum samples must be uncontaminated with fungal spores and/or bacteria. Transport and store samples in sealed tubes, unexposed to air. Samples may be stored at +2-8°C for 24 hours prior to testing. For longer storage, store the serum at -70°C.
Serum samples can be subjected to a maximum of 3 freezing / thawing cycles. Previously frozen specimens should be thoroughly mixed after thawing prior to testing.
The results are not affected by samples containing 90 g/L of albumin, 200 mg/L of bilirubin, lipemic samples containing the equivalent of 360 g/L of triolein (triglyceride) or hemolyzed samples containing 200 g/L of hemoglobin.
Do not decomplement sera.

**10- PROCEDURE**
**Materials provided**
See REAGENTS section.
**Materials required but not provided**

1. Sterile distilled or deionized water, for dilution of Concentrated Washing Solution.
2. Absorbent paper.
3. Disposable gloves.
4. Protective glasses.
5. Sodium hypochlorite (bleach) and sodium bicarbonate.
6. Pipettes or multipipettes, adjustable or fixed, to measure and dispense 50 µL, 100 µL, 300 µL, and 1000 µL.
7. 1.5 mL polypropylene microcentrifuge tubes with airtight stoppers, able to support heating to 120°C (heat block) or 100°C (boiling water bath):
   - Screw cap tubes: 1.5 mL conical tubes, Bio-Rad Cat. # 224-0010 or equivalent.
   - Snap cap tubes: EZ Micro Test Tubes, 1.5 mL, Bio-Rad Cat. # 223-9480 or equivalent.
   - Micro-tube cap locks: VWR Cat. # 6054001 or equivalent. These locks securely seal snap cap tubes by preventing caps from opening during temperature and pressure changes and also allow tubes to be easily lifted out of heat block or boiling water bath.
8. Laboratory bench centrifuge for 1.5 mL polypropylene tubes capable of obtaining 10,000g.
9. If heat block is used for the treatment of the sera:
   - Heat block. The following heat block models are recommended:
     - 1 block model: Grant Cat. # QBD1 (VWR Cat. # 460-0074)
     - 2 block model: Grant Cat. # QBD2 (VWR Cat. # 460-0076)
   - Block for heat block: both heat blocks (QBD1 and QBD2) must be used with Grant block Cat. # QB-E1 (VWR Cat. # 460-8517)
   If boiling water bath is used for the treatment of the sera:
   - Round, floating micro-centrifuge rack for 1L beaker.
   - Boiling water bath at 100°C
10. Vortex agitator.
11. Microplate incubator at 37 ± 1°C.
12. Semi-automated or automated microplate washer.
14. Contaminated waste container.

**Procedural Comments**

Negative, Positive Controls and range points must be tested on each run to validate the test results.
Preparation of the range points

**Quantitative mode:**

<table>
<thead>
<tr>
<th></th>
<th>For 1 series</th>
<th>For 6 series (*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R3</td>
<td>R4</td>
</tr>
<tr>
<td>R3 Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R4 2 ng/mL</td>
<td>300 µL</td>
<td>2000 µL</td>
</tr>
<tr>
<td>Calibrator Serum</td>
<td>150 µL</td>
<td>1000 µL</td>
</tr>
<tr>
<td></td>
<td>75 µL</td>
<td>500 µL</td>
</tr>
<tr>
<td>2 ng/mL point</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1 ng/mL point</td>
<td>150 µL</td>
<td>1000 µL</td>
</tr>
<tr>
<td>0.5 ng/mL point</td>
<td>225 µL</td>
<td>1500 µL</td>
</tr>
<tr>
<td>0.25 ng/mL point</td>
<td>262.5 µL</td>
<td>1750 µL</td>
</tr>
<tr>
<td></td>
<td>37.5 µL</td>
<td>250 µL</td>
</tr>
</tbody>
</table>

(*): a volume of reagent corresponding to 6 series can be prepared in advance: for each range point, divide into 300 µL aliquots and store at -20°C. Bring reagents to room temperature (18-25°C) before use.

**Qualitative mode:**

Prepare the 0.5 ng/mL (calibrator serum) and 0.25 ng/mL range points as indicated in the previous table.

**Treatment of the sera**

All control sera: negative (R3), positive (R5) and the 2.0, 1.0, 0.5 and 0.25 ng/mL range points must be processed at the same time as test samples:

1. Pipette 300 µL of each test serum, control and range point into individual 1.5 mL polypropylene tubes.
2. Add 100 µL of Serum Treatment Solution (R7) to each tube.
3. Mix tubes thoroughly by vigorous mixing or vortexing to mix thoroughly. Tightly close the tube to prevent opening during heating. Do not pierce the stopper.
4. **Heat block option:**
   Heat tubes for 6 minutes in a heat block at 120°C. Tubes must be placed in the block only when the prescribed temperature is reached. (*)
   **OR**
   **Water bath option:**
   If using a boiling water bath: heat tubes for 3 minutes at 100°C. Tubes must be placed in the water bath only when the prescribed temperature is reached. (*)
5. Carefully remove hot tubes from the heat block or the boiling water bath and place in a centrifuge. Centrifuge tubes at 10,000 x g for 10 minutes. The supernatant is used for detection of the mannan antigen.

6. Test the supernatants using the following procedure. After treatment of the serum, the test must be performed as rapidly as possible. If analysis of the results indicates retesting is required, another aliquot of serum must be treated for testing.

(*) Strict compliance with the prescribed temperature and the prescribed turn-around time as well as use of recommended materials are essential for success of the test. Do not rely on the temperature displayed by the apparatus, but check that the temperature complies with specifications by using a calibrated thermometer which will be fitted into a tube containing mineral oil: 120°C must be reached inside the tube in a heat block and 100°C in a boiling water bath.

**NB:**

- All serum samples treated according to this procedure can be used to perform the Platelia™ *Aspergillus* EIA test, as the serum treatment procedures are identical for the two tests.
- Do not store sera (controls, range points and test samples) after treatment.

**EIA Procedure**

Strictly comply with the proposed protocol. Comply with Good Laboratory Practice.

1. Bring reagents to room temperature (18-25°C) for at least 15 minutes before use.
2. Prepare Working Washing Solution, Substrate-Chromogen Reaction Solution, Negative and Positive Controls and range points.
3. Prepare a chart for identification of test sera, controls and range points in the microplate.

**Quantitative mode:**

Control sera and range points (0.25, 0.50, 1.0 and 2.0 ng/mL) can be placed according to the following plan:

- A1: Negative Control Serum (R3)
- B1: 0.25 ng/mL range point
- C1: 0.50 ng/mL range point
- D1: 1.0 ng/mL range point
- E1: 2.0 ng/mL range point (R4)
- F1: Positive Control Serum (R5)
- G1: Positive Control Serum (R5)
Qualitative mode:
Control sera and 0.5 ng/mL and 0.25 ng/mL range points can be placed according to the following plan:
- A1: Negative Control Serum (R3)
- B1: 0.25 ng/mL range point
- C1: 0.5 ng/mL range point (Calibrator Serum)
- D1: 0.5 ng/mL range point (Calibrator Serum)
- E1: Positive Control Serum (R5)

4. Remove the plateholder and microwell strips (R1) from the plate pouch. Return any strips that will not be used to the pouch, with the desiccant, and reseal the pouch.

5. Mix the contents of the Conjugate bottle (R6) by inverting before use. Add 50 µL of Conjugate to each well. Next, add 50 µL of treated serum supernatant to each well, as designated above. Do not add serum samples to the wells before the Conjugate.

6. Cover plate with plate sealer, or other means to prevent evaporation, ensuring that entire surface is covered and watertight.

7. Incubate the microplate in a dry microplate incubator for 90 ± 5 minutes at 37°C (± 1°C).

8. Remove the plate sealer. Aspirate the contents of all wells into a waste container (containing sodium hypochlorite). Wash the plate 5 times, using a minimum of 370 µL of Working Washing Solution. After the last wash, invert the microplate and gently tap on absorbent paper to remove remaining liquid.

9. Rapidly add 200 µL of Substrate-Chromogen Reaction Solution (R8 + R9) to each well, avoiding exposure to bright light.

10. Incubate microplate in the dark at room temperature (18-25°C) for 30 ± 5 minutes. Do not use adhesive film during this incubation step.

11. Add 100 µL of Stopping Solution (R10) to each well, utilizing the same order for addition of Substrate-Chromogen Reaction Solution. Mix well.

12. Thoroughly wipe the bottoms of each plate.

13. Read the optical density of each well at 450 nm (reference filter of 620/630 nm). Microplates must be read within 30 minutes of addition of Stopping Solution.
11- QUALITY CONTROL (VALIDITY CRITERIA)

Quantitative mode
Use control sera and range points on each microplate for each test. The following criteria must be satisfied to validate the test procedure:

- Optical density value:
  \[ 0.300 < \text{OD of the 0.5 ng/mL range point} < 1.100 \]

- Ratios:
  \[ \frac{\text{OD (2.0 ng/mL range point)}}{\text{OD (0.5 ng/mL range point)}} > 2.1 \]
  \[ \frac{\text{OD (1.0 ng/mL range point)}}{\text{OD (0.5 ng/mL range point)}} > 1.25 \]
  \[ \frac{\text{OD (0.25 ng/mL range point)}}{\text{OD (0.5 ng/mL range point)}} < 0.85 \]
  \[ \frac{\text{OD (R3)}}{\text{OD (0.25 ng/mL range point)}} \leq 0.80 \]

- Mannan concentration:
  Concentration of R5 equal to the concentration indicated on the vial ± 20%.

Qualitative mode

- Calculation of the Cut-Off Value (CO)
  Add the OD values obtained for each well containing the 0.5 ng/mL range point and divide by 2.

- Validity criteria
  \[ 0.300 < \text{CO} < 1.100 \]
  \[ \text{OD R5} > \text{CO} \]
  \[ \text{OD R3} < 0.7 \text{ CO} \]
  \[ 0.25 \text{ ng/mL range point OD} < 0.85 \text{ CO} \]

12- INTERPRETATION OF RESULTS

QUANTITATIVE MODE

Establishing the standard curve
The standard curve is plotted with 4 range points, 2.0, 1.0, 0.5 and 0.25 ng/mL, prepared from the Calibrator Serum R4. Positive (R5) and negative (R3) controls are not included in the curve. To obtain maximum precision, plot a sigmoid curve with extrapolation by plotting mannan concentration in ng/mL on the X-axis (logarithmic scale) and optical density on the Y-axis (linear scale).
If the plate reader does not allow this type of representation, plot a curve connecting the various range points.
**Determination of the mannan concentration in test sera**

The calibration curve can be used to determine the mannan antigen concentration, expressed in ng/mL, for each test sample.

**Interpretation of the results**

- Sera with a mannan concentration strictly less than 0.25 ng/mL \((C < 0.25)\) are considered to be "negative" for the presence of mannan antigen.
- Sera with a mannan concentration between 0.25 and 0.5 ng/mL \((0.25 \leq C < 0.5)\) are considered to be "intermediate" for the presence of mannan antigen.
- Sera with a mannan concentration greater than or equal to 0.5 ng/mL \((C \geq 0.5)\) are considered to be "positive" for the presence of mannan antigen.
- The range points used to plot the calibration curve do not allow precise determination of concentrations greater than 2.5 ng/mL. The test should be repeated after dilution of the serum to 1/5 in negative serum (R3) in order to obtain a more precise determination of the concentration of strongly positive sera.

**Note:** The Platelia™ *Candida* Ag is intended to be used as an aid in the diagnosis of Invasive Candidiasis. Positive results obtained with the Platelia™ *Candida* Ag should be considered in conjunction with other diagnostic procedures such as microbiological culture, histological examination of biopsy samples and radiographic evidence.

Regular screening of high-risk patients is recommended to increase the sensitivity and early positivity of the test.

**QUALITATIVE MODE**

**Interpretation of the results**

- Sample OD < 0.25 ng/mL range point OD: sera are considered to be "negative" for the presence of mannan antigen.
- Sample OD \(\geq 0.25\) ng/mL range point OD \(\leq\) sample OD < CO: sera are considered to be "intermediate" for the presence of mannan antigen.
- Sample OD \(\geq\) CO: sera are considered to be "positive" for the presence of mannan antigen.

**Note:** the Platelia™ *Candida* Ag is intended to be used as an aid in the diagnosis of Invasive Candidiasis. Positive results obtained with the Platelia™ *Candida* Ag should be considered in conjunction with other diagnostic procedures such as microbiological culture, histological examination of biopsy samples and radiographic evidence.
Regular screening of high-risk patients is recommended to increase the sensitivity and early positivity of the test.

**NB:**
To determine the intensity of positive results, it is possible to calculate an index (I):

\[ I = \frac{\text{positive serum } \text{OD}}{\text{CO}} \]

This index allows to express results in semi-quantitative mode.

**13- LIMITATIONS OF THE PROCEDURE**

1. A negative test cannot rule out the diagnosis of Invasive Candidiasis due to the very low concentration and rapid elimination of mannan during infection.

2. A negative test for mannan antigen must also be interpreted in conjunction with the results of anti-mannan antibody tests: even in case of Invasive Candidiasis, the antigen is more difficult to detect in patients tested positive for anti-mannan antibodies (refer to Chapter-14. Specific Performance Characteristics).

3. The performance of detection of mannan antigen in serum is related to the frequency of tests performed in the patient. Regular monitoring of high-risk patients and screening for anti-mannan antibodies are recommended to increase the sensitivity and early positivity of the test.

4. The Platelia™ *Candida* Ag procedure and the interpretation of results must be followed when testing samples for the presence of mannan antigen. The user of the kit is advised to read the package insert carefully prior to conducting the test. In particular, the test procedure must be carefully followed for sample and reagent pipetting, plate washing, and timing of the incubation steps.

5. Failure to add specimen or reagent as instructed in the procedure could result in a falsely negative test. **Repeat testing of additional samples should be considered where there is clinical suspicion of Invasive Candidiasis or procedural error.**

6. Contamination of negative patient specimen wells by positive control/patient specimen wells is possible if the contents of one well spill over into another well due to rough handling of the microplate or poor pipetting technique while adding reagents.

7. There has been report of cross-reaction in patients receiving an infusion with certain batches of hydroxyethylstarch plasma expanders (such as Hesteril 6%), used in the treatment of circulatory failure: hypovolaemia, hemorrhagic shock, septic shock.
14-SPECIFIC PERFORMANCE CHARACTERISTICS

14.1 QUANTITATIVE MODE

A) Reproducibility Studies

- Intra-assay variability:
  Four sera (one negative, one intermediate at 0.48 ng/mL and two positive sera with concentrations of 0.69 ng/mL and 1.39 ng/mL) were tested in thirty replicates. The coefficients of variation were 3.1%, 6.9%, 9.1% and 7.0%, respectively.

- Inter-assay variability:
  Eight negative sera and four positive sera were tested on five series performed over a period of five weeks. The coefficients of variation were < 20% for the concentrations of positive sera and < 13% for the OD of negative sera.

B) Clinical Testing

SPECIFICITY

The specificity results are summarized in the following table:

<table>
<thead>
<tr>
<th>Patient category</th>
<th>Number of patients (nb of sera)</th>
<th>C &lt; 0.25</th>
<th>0.25 ≤ C &lt; 0.5</th>
<th>C ≥ 0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not hospitalized</td>
<td>184 (184)</td>
<td>183 (99.5%)</td>
<td>1 (0.5%)</td>
<td></td>
</tr>
<tr>
<td>Hospitalized, not colonized, with:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Crohn’s disease</td>
<td>151 (200)</td>
<td>140 (92.8%)</td>
<td>7 (4.6%)</td>
<td>4 (2.6%)</td>
</tr>
<tr>
<td>- Ulcerative colitis</td>
<td>52 (52)</td>
<td>49 (98.1%)</td>
<td>3 (1.9%)</td>
<td></td>
</tr>
<tr>
<td>- Aspergillosis</td>
<td>43 (43)</td>
<td>41 (95.4%)</td>
<td>1 (2.3%)</td>
<td>1 (2.3%)</td>
</tr>
<tr>
<td>- Pneumocystosis</td>
<td>26 (35)</td>
<td>23 (88.5%)</td>
<td>1 (3.8%)</td>
<td>2 (7.7%)</td>
</tr>
<tr>
<td>- Cryptococcosis</td>
<td>4 (4)</td>
<td>4 (100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospitalized colonized by Candida</td>
<td>41 (160)</td>
<td>35 (85.3%)</td>
<td>4 (9.8%)</td>
<td>2 (4.9%)</td>
</tr>
</tbody>
</table>

SENSITIVITY

Clinical testing to evaluate the sensitivity of the Platelia™ Candida Ag was conducted at three university hospitals located in France. The study was conducted retrospectively using a total of 366 serum samples collected from 106 patients from different hospital wards: surgery, hematology, intensive care, burns...

Candida yeasts were isolated from blood cultures or deep samples of these patients.8,9
In this patient population, the overall sensitivity of Platelia™ *Candida* Ag was 51.5% (excluding sera with a mannan antigen concentration between 0.25 and 0.5 ng/mL which are considered to be intermediate for the presence of mannan antigen: 6.6% of patients).

Depending on the isolated *Candida* species, sensitivity varies as shown in the table below:

<table>
<thead>
<tr>
<th>Species of <em>Candida</em> isolated</th>
<th>Number of patients (nb of sera)</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C ≥ 0.5</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>10 (72)</td>
<td>70.0 %</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>12 (31)</td>
<td>58.3 %</td>
</tr>
<tr>
<td>C. albicans</td>
<td>64 (208)</td>
<td>52.5 %</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>2 (5)</td>
<td>50.0 %</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>10 (29)</td>
<td>37.5 %</td>
</tr>
<tr>
<td>C. krusei</td>
<td>8 (21)</td>
<td>20.0 %</td>
</tr>
</tbody>
</table>

Sensitivity depends on the number and frequency of samples. These clinical studies also demonstrated the value of combining the detection of mannan antigen with Platelia™ *Candida* Ag and anti-mannan antibodies with Platelia™ *Candida* Ab/Ac/Ak.

Patients can be classified into 2 significantly different populations (Chi-square test, p < 0.0005), as illustrated by the results presented in the following table:

- Negative anti-mannan antibody patients: sensitivity of Platelia™ *Candida* Ag is 78.8% in the presence of *C. albicans* infections and 66.6% for combined *Candida* species.
- Positive anti-mannan antibody patients: antigen mannan is more difficult to detect.

<table>
<thead>
<tr>
<th>Infecting species (nb of patients)</th>
<th>Anti-mannan Ab negative patients</th>
<th>Anti-mannan Ab positive patients</th>
<th>All patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined <em>Candida</em> species (106)</td>
<td>66.6 %</td>
<td>17.5 %</td>
<td>51.5 %</td>
</tr>
<tr>
<td><em>C. albicans</em> (64)</td>
<td>78.8 %</td>
<td>16.1 %</td>
<td>52.5 %</td>
</tr>
</tbody>
</table>

Interpretation of Platelia™ *Candida* Ag testing must therefore include information on patient immune status to *Candida* mannan.

The combined serological detection of mannan antigen and anti-mannan antibodies demonstrates a sensitivity of 84.8% (excluding the 6.6% of patients with an intermediate concentration of mannan antigen).
However, some patients demonstrate a positive response to the two markers at various times during the same episode of infection. Prognosis of the infection may be linked to the balance between mannanemia and antimannan antibody response\textsuperscript{13}.

14.2 QUALITATIVE MODE

A) Reproducibility Studies

- Intra-assay variability:
  Four sera (one negative, one intermediate with index = 0.95 and two positive sera with index = 1.24 and 2.00) were tested in thirty replicates. The percent coefficients of variation (%CV) were 3.1\%, 7.2\%, 5.9\% and 5.9\%.

- Inter-assay variability:
  Ten positive sera and four intermediate sera were tested on six series. The percent coefficients of variation (%CV) were $< 15\%$ for the concentrations of positive and intermediate sera.

B) Clinical Testing

SPECIFICITY - SENSITIVITY

Considering that concentrations $\geq 0.5$ ng/mL or $< 0.25$ ng/mL obtained on sera in quantitative mode, correspond respectively to positive and negative sera in qualitative mode, performance characteristics of the test in qualitative mode are equivalent to the ones described in chapter “Quantitative mode”.

15- QUALITY CONTROL OF THE MANUFACTURER

All manufactured reagents are prepared according to our Quality System, starting from reception of raw material to the final commercialization of the product.

Each lot is submitted to quality control assessments and is only released to the market, after conforming to pre-defined acceptance criteria. The records relating to production and control of each single lot are kept within Bio-Rad.


(US) - The other languages which are required in conformity to the European Directive can be obtained from your local Bio-Rad agent.

(F) - Les autres langues requises par la Directive Européenne sont disponibles auprès de votre représentant Bio-Rad local.

(E) - Los otros idiomas que se requieren para la conformidad de la Diretiva Europea puede ser obtenida en su oficina local Bio-Rad.

(I) - Le altre lingue che sono richieste in conformità con le Direttive Europee possono essere ottenute dal locale agente Bio-Rad.

(D) - Die anderen Sprachen, die in Übereinstimmung mit der europäischen IVD Direktive benötigt werden, erhalten Sie über Ihre lokale Bio-Rad Niederlassung.

(P) - As restantes línguas, obrigatórias em conformidade com a Directiva Europeia, podem ser obtidas através da subsidiária Bio-Rad mais próxima de si.

(S) - Övriga språk som kräve i enlighet med EG-direktivet kan erhållas från din lokala Bio-Rad-representant.

(DK) - De øvrige sprog som kræves i henhold til EU direktiv kan fås ved henvendelse til den lokale Bio-Rad leverandør.

(GR) - Οι υπόλοιπες γλώσσες που απαιτούνται για συμμόρφωση της Ευρωπαϊκής διοίκησης μπορείτε να τις προκαθορίσετε από τον τοπικό σας αντιπρόσωπο Bio-Rad.

(PL) - Tłumaczenie w innych językach które są wymagane w Dyrektywie Unijnej może być otrzymane od lokalnego przedstawiciela firmy Bio-Rad.

(LT) - Vertinimas, reikalingas pagal Europos sajungos direktyvos reikalavimus, j kitas kalbas galite gauti iš vietinio Bio-Rad atstovo.

(H) - A leírás az Európai Irányelv által előírt egyéb nyelven hozzáférhető a Bio-Rad helyi kirendeltségeinél.

(EST) - Teised vastavalt Euroopa Direktiivile nõutavad kohalikud Bio-Radi edasimüüja käest.

(SK) - Ostatné jazykove verzie, ktoré sú vyžadované v zhode s Európskou direktívou, možno obdržať od vášho lokálneho zástupcu Bio-Rad.

(CZ) - Další jazykové verze vyžadované ve shodě s evropskou direktivou jsou k dispozici u lokálního zastoupení firmy Bio-Rad.