DETECTION OF THE MANNAN ANTIGEN OF CANDIDA IN HUMAN SERUM OR PLASMA BY IMMUNOENZYMATIC ASSAY
1- **INTENDED USE**
Platelia™ *Candida* Ag Plus is an immunoenzymatic sandwich microplate assay for the detection of the circulating mannan *Candida* antigen in human serum or plasma.

2- **INDICATIONS FOR USE**
A diagnosis of invasive candidiasis must be based on a combined detection of antibodies and circulating antigens. 13
Platelia™ *Candida* Ag Plus (code 62784) is a test which used in association with Platelia™ *Candida* Ab Plus (code 62785) helps to improve the earliness 11 and the sensitivity of this diagnosis as a part of a complete diagnostic approach combining intrinsic and iatrogenic risk factors as well as clinical and mycological data 10, 13, 14. This test combination is also an integral part of a clinical and laboratory patient monitoring process as an aid in therapeutic decisions. 8

3- **CLINICAL VALUE**
*Candida* infections rank as the first cause of nosocomial fungal infections; candidemias are the fourth cause of nosocomial blood stream infections (BSI). 12, 15, 16
Invasive candidiasis represent the most serious forms of *Candida* infections with a mortality rate ranging from 30 to 70% in immunosuppressed patients. Their diagnosis is still difficult due to the lack of specificity of the clinical symptoms and the poor sensitivity of the blood culture. The diagnosis of invasive candidiasis, leading to initiation of appropriate treatment, is usually based on a combination of data 2. In this context, the diagnosis of systemic candidiasis must associate serological techniques with direct mycological methods. The detection of circulating antigens in serum or plasma appears to improve the diagnosis in patients at risk of invasive candidiasis 10, 13, 14. The principal risk factors include neutropenia after chemotherapy or immunosuppressive treatment (in cancer, oncohematological and transplant patients) 4,10, wide spectrum antibiotherapy, venous catheters, parenteral nutrition, renal dialysis, implanting of prosthesis (in patients hospitalized in medical and surgical intensive care units). 5, 13, 16
Amongst *Candida* antigens, mannan 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 biomarkers for the diagnosis of invasive candidiasis.
The regular monitoring of at-risk patients, combining the detection of circulating mannan antigen and anti-mannan antibodies, is an aid to the diagnosis of invasive candidiasis. 9, 10, 13
4- PRINCIPLE OF THE PROCEDURE

Platelia™ Candida Ag Plus is a one-stage immunoenzymatic sandwich microplate assay, allowing the detection of the circulating mannan Candida antigen in human serum or plasma. The assay uses the rat monoclonal antibody (MAb), EBCA-1, which is directed against Candida α 1-5 oligomannosides, and has been characterized in previous studies 13, 14. The EBCA1 MAb is used to:

- Coat the microplate wells and bind the mannan antigen,
- Detect the antigen bound to the sensitized microplate (conjugate reagent: peroxidase labelled MAb).

Serum or plasma samples are heat-treated in the presence of EDTA in order to dissociate the immune complexes and to precipitate the serum proteins which could possibly interfere with the immunoassay reaction. The treated samples of serum or plasma and the conjugate are added to the wells of the microplate coated with the anti-mannan monoclonal antibody.

After incubation at 37°C, the strips are washed in order to remove any unbound material. If there is circulating mannan antigen in the human sample, a complex: anti-mannan MAb – mannan Ag - anti-mannan MAb / peroxydase, is formed.

Next, the chromogen solution containing the peroxidase substrate is added and incubated at room temperature, allowing the detection of any complex bound to the microplate well.

The enzymatic reaction is stopped by the addition of 1N sulphuric acid. The absorbance (optical density) of human samples and calibrator is determined with a spectrophotometer set at 450/620 nm wavelength.

5- REAGENTS

Reagents are supplied in sufficient quantity to perform 96 determinations in a maximum of 9 batches.

Refer to the indications mentioned on the box labels for the storage conditions and expiry date of the reagents.

Bring all reagents to room temperature (+18-30°C) before use. Return all the reagents back to +2-8°C immediately after use. Return the unused strips to their original bag and reseal carefully. Do not remove the desiccant.
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<thead>
<tr>
<th>Component</th>
<th>Contents</th>
<th>Quantity</th>
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<tbody>
<tr>
<td>R1 Microplate</td>
<td>Microplate: - 96 wells (12 strips each of 8 wells) sensitized with the anti-mannan monoclonal antibody EBCA-1.</td>
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<tr>
<td>R2 Concentrated Washing Solution (20x)</td>
<td>Concentrated washing solution (20 x) : - Tris-NaCl buffer (pH 7.4) - 2% Tween® 20 - Preservative: &lt; 1.5% ProClin™ 300</td>
<td>1 x 70 ml</td>
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<tr>
<td>R3 Calibrator 0</td>
<td>Calibrator 0 pg/ml (ready to use): - Tris-NaCl-Trehalose buffer, not containing any purified mannan of Candida albicans - Preservative: &lt; 1.5% ProClin™ 300</td>
<td>1 x 2.0 ml</td>
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<tr>
<td>R4a Calibrator 62.5</td>
<td>Calibrator 62.5 pg/ml (ready to use): - Tris-NaCl-Trehalose buffer - Purified mannan of Candida albicans - Preservative: &lt; 1.5% ProClin™ 300</td>
<td>1 x 2.0 ml</td>
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<tr>
<td>R4b Calibrator 125</td>
<td>Calibrator 125 pg/ml (ready to use): - Tris-NaCl-Trehalose buffer - Purified mannan of Candida albicans - Preservative: &lt; 1.5% ProClin™ 300</td>
<td>1 x 2.0 ml</td>
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<tr>
<td>R4c Calibrator 250</td>
<td>Calibrator 250 pg/ml (ready to use): - Tris-NaCl-Trehalose buffer - Purified mannan of Candida albicans - Preservative: &lt; 1.5% ProClin™ 300</td>
<td>1 x 2.0 ml</td>
</tr>
<tr>
<td>R4d Calibrator 500</td>
<td>Calibrator 500 pg/ml (ready to use): - Tris-NaCl-Trehalose buffer - Purified mannan of Candida albicans - Preservative: &lt; 1.5% ProClin™ 300</td>
<td>1 x 2.0 ml</td>
</tr>
<tr>
<td>R0 Negative Control</td>
<td>Negative Control: - Human Serum negative for mannan - Preservative: &lt; 1.5% ProClin™ 300</td>
<td>2 x 1.5 ml</td>
</tr>
<tr>
<td>R5 Positive Control</td>
<td>Positive Control: - Human Serum containing mannan - Preservative: &lt; 1.5% ProClin™ 300</td>
<td>2 x 1.5 ml</td>
</tr>
<tr>
<td>R6 Conjugate</td>
<td>Conjugate (ready to use): - Monoclonal anti-mannan antibody, labelled with peroxidase - Bromocresol purple - Preservative: &lt; 1.5% ProClin™ 300</td>
<td>1 x 17 ml</td>
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<tr>
<td>R7 Sample Treatment Solution</td>
<td>Sample Treatment Solution (ready to use): - Acid solution of EDTA without preservative</td>
<td>1 x 10.5 ml</td>
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</table>
6- WARNINGS FOR USERS

1. For *in vitro* diagnostic use only.
2. For professional use only.
3. The use of this test with samples other than human serum or plasma is not recommended.
4. The R0 Negative Control and the R5 Positive Control are prepared from human serum that has been tested and found non-reactive for anti-HIV-1, anti-HIV-2 and anti-HCV antibodies and also for HBs antigen with CE marked tests. However, all the reagents must be handled as if they were potentially infectious. All tests should be conducted in accordance with the OSHA Standard on Bloodborne Pathogens, Biosafety Level 2 or other appropriate biosafety practices.
5. Wear protective clothing, including lab coat, eye/face protection and disposable gloves (synthetic, non-latex gloves are recommended) and handle the kit reagents and the patient samples with the requisite Good Laboratory Practices (GLP). 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.
9. Biological spills not containing acid should be wiped thoroughly with an effective disinfectant. Disinfectants that can be used include (but not limited to) a solution of 10% bleach (0.5% solution of sodium hypochlorite), of 70% ethanol or 0.5% Wescodyne Plus™. Materials used to wipe up spills may require biohazardous waste disposal.

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

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<th>Component</th>
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<tr>
<td>R9</td>
<td>Chromogen TMB Solution (ready to use): - Solution de 3,3',5,5'- tetramethylbenzidine (&lt; 0,1%), H₂O₂ (&lt;1,0%)</td>
<td>1 x 28 ml</td>
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<tr>
<td>R10</td>
<td>Stopping Solution (ready to use): - sulphuric acid solution 1N</td>
<td>1 x 28 ml</td>
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<tr>
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<td>Adhesive plate sealers</td>
<td>4</td>
</tr>
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</table>
10. Spills containing acid should be appropriately absorbed (wiped up) or neutralized with sodium bicarbonate, and the area rinsed and wiped dry; if it contained biohazardous material, wipe the area with one of the chemical disinfectants.

11. Dispose of all the specimens and materials used to perform the test as though they contain an infectious agent. Laboratory chemical and biohazardous wastes must be handled and discarded in accordance with all current regulations.

12. **CAUTION:** The following is a list of potential chemical hazards contained in some kit components (refer to section 5 - REAGENTS)

**CAUTION:** Some of the reagents contain ProClin™ 300 < 1.5%

_For risks and security recommendations refer to the table at the end of the package insert._

13. The Material Safety Data Sheet (MSDS) is available on request.

7- **PRECAUTIONS FOR USERS**

1. FROZEN SERUM OR PLASMA 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. Do not mix reagents from other kits that have different lot numbers, with the exception of the Washing Solution (R2, identification*: 20x coloured green), the Chromogen (R9, identification*: TMB coloured turquoise) and the Stopping Solution (R10, identification*: 1N coloured red), provided that these reagents are strictly equivalent and that the same lot number is used within a given test run.

   **NOTE:** the Washing Solution (R2, identification*: 20x coloured green), the Chromogen (R9, identification*: TMB coloured turquoise) and the Stopping Solution (R10, identification*: 1N coloured red), provided that these reagents are strictly equivalent and that the same lot number is used within a given test run.

   **NOTE:** Washing Solution (R2 identification*: 20X coloured green) cannot be mixed with Washing Solution (R2 identification*: 10X coloured blue) supplied in the Bio-Rad reagent kits.

   * on the vial label

4. Bring all reagents to room temperature (+18 to +30°C) for at least 30 minutes before use.

5. Use glassware thoroughly washed and rinsed with distilled water or, preferably disposable material.

6. Check pipettes and other equipment for accuracy and correct operation.
7. Carefully reconstitute or dilute the reagent R2 avoiding any contamination.
8. The enzymatic reaction is very sensitive to metal or metal ions. Consequently, do not allow any metal element to come into contact with the various solutions containing the conjugate or the substrate solution.
9. For manual pipetting of calibrators, controls and specimens, use individual pipette tip to prevent carryover of samples.
10. 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 must be carried out with a microplate washer.
11. Do not allow the microplate to dry between the end of the wash cycle and addition of the reagents.
12. Do not use the same container to distribute the conjugate and the development solution.
13. Avoid exposing the Chromogen TMB solution to strong light during storage or incubation. Do not allow the chromogen solution to come into contact with an oxidizing agent.
14. Chromogen solution (R9) must be colorless. The appearance of a blue color indicates the reagent should not be used and must be discarded.
15. Avoid contact of the stopping solution with any oxidizing agent, metal or metallic ions.
16. Do not pour any unused Conjugate back into the original container.

8- REAGENT PREPARATION AND STORAGE

Microplate (R1)
Each carrier tray containing 12 strips is packaged in a bag. Cut the bag with scissors, just below the seal. Open the bag and take the carrier tray out. Replace the carrier tray containing the unused strips in the original bag. Carefully reseal the bag and return it to +2-8°C.
After opening the vacuum-packed bag, the microwell strips are stable for 8 weeks when stored at +2-8°C in their carefully resealed original bag in the presence of the enclosed desiccant.

Washing Solution (R2)
Prepare the working Washing Solution by diluting the Concentrated Washing Solution 20 times in distilled water: 50 ml of R2 in 950 ml of distilled water. Use 400 ml of working Washing Solution for one complete 12 strip microplate, excluding dead volume due to the equipment used). The working Washing Solution can be stored for 14 days a +2-30°C.
After opening, the Concentrated Washing Solution stored at +2-30°C, is stable until the expiration date indicated on the label, if there is no contamination.
Calibrator 0 (R3), Calibrator 62.5 (R4a), Calibrator 125 (R4b), Calibrator 250 (R4c), Calibrator 500 (R4d):
The calibrators are ready to use.
After opening, these reagents stored at +2-8°C, are stable for 8 weeks, if there is no contamination.

Negative Control (R0), Positive Control (R5):
The Negative Control (R0) and the Positive Control (R5) must be heat-treated with the EDTA acid solution (R7) as patient specimens, in order to also be a monitor of the treatment.
After opening, the reagents stored at +2-8°C, are stable for 8 weeks, in the absence of contamination.

Conjugate (R6), Sample Treatment Solution (R7), TMB Chromogen Solution (R9):
These reagents are ready to use.
After opening, these reagents stored at +2-8°C, are stable for 8 weeks in the absence of contamination.

Stopping Solution (R10):
This reagent is ready to use.
After opening, this reagent stored at +2-8°C, is stable until the expiration date indicated on the label, in the absence of contamination.

9- SPECIMENS
1. The test is performed on serum or plasma specimens collected on EDTA, heparin or citrate anticoagulants.
2. Comply with the following guidelines for heat-treating, processing and storing blood samples:
   • Collect a blood sample according to standard laboratory procedures.
   • For serum, allow samples to clot completely before centrifugation.
   • Keep the tubes stoppered at all times.
   • After centrifugation, extract the serum or plasma and store it in a sealed tube.
   • The specimens can be stored at +2-8°C if the test is performed within 5 days.
   • If the test cannot be performed within 5 days, freeze the samples at -20°C (or -80°C.)
   • Serum or plasma samples can be subjected to a maximum of 5 freezing/thawing cycles. Previously frozen specimens should be thoroughly mixed after thawing prior to testing.
3. The results are not affected by samples containing 60 g/l of human albumin or 120 g/l of total proteins or 200 mg/l of unconjugated bilirubin or 200 mg/l of conjugated bilirubin, lipemic samples containing the equivalent of 30 g/l of triolein (triglyceride), or 5 g/l of cholesterol or hemolyzed samples containing 2 g/l of hemoglobin.

4. Do not heat sera or plasma.

10- PROCEDURE

Materials provided
See REAGENTS section.

Material required but not provided
1. Sterile distilled or deionized water, for dilution of the Concentrated Washing Solution.
2. Absorbent paper.
3. Disposable gloves.
4. Goggles or protective glasses.
5. Sodium hypochlorite (bleach) and sodium bicarbonate.
6. Pipettes or multi-pipettes, whether automatic or semi-automatic, adjustable or preset, pipettes or multi-pipettes, to measure and dispense 10 µl to 1000 µl, and 1 ml, 2 ml and 10 ml.
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 ref. 224-0010 or equivalent.
   OR
   • Snap cap tubes: EZ Micro Test Tubes, 1.5 ml, Bio-Rad ref. 223-9480 or equivalent.
8. Micro-tube cap locks (VWR ref. 6054001 or equivalent). These locks securely seal snap cap tubes by preventing caps from opening during temperature or pressure changes. A small handle allows the microtube to be easily lifted out of the heating block or the boiling water bath, thus avoiding any risk of burning.
9. Laboratory bench centrifuge for 1.5 ml polypropylene tubes capable of obtaining 10,000g.
10. If heat block is used for the treatment of the sera:
   • Heat block. The following heat block models are recommended:
      - single block model: Grant ref. QBD1 (VWR ref. 460-0074)
      - two block model: Grant ref. QBD2 (VWR ref. 460-0076)
   • Block for heat blocks: both heat blocks (QBD1, QBD2) must be used with Grant block ref. QB-E1 (VWR ref. 460-8517).
If a boiling water bath is used for the treatment of the sera:
• Floating micro-centrifuge rack
• Boiling water bath which can be thermostatically set at 100°C.

11. 25 ml, 50 ml, 100 ml and 1000 ml graduated test tubes.
13. Vortex agitator.
14. Microplate incubator which can be thermostatically set at 37 ± 1°C (*).
15. Automated or semi-automated microplate washer (*).
16. Microplate reader equipped with 450 nm and 620 nm filters (*).

(*) Consult us for detailed information about the equipment recommended by our technical services.

Treatment of samples
The Negative Control (R0) and the Positive Control (R5) must be treated at the same time as all the patient samples by adding 100 µl of treatment solution (R7). R3, R4a, R4b, R4c and R4d Calibrators which are ready for use must not undergo this treatment.

1. Pipette 300 µl of each patient sample, of the Negative Control (R0) and of the Positive Control (R5) into individual 1.5 ml polypropylene tube.
2. Add 100 µl of the Sample Treatment Solution (R7) to each tube.
3. Mix tubes thoroughly by vigorous mixing or vortexing. Tightly close the tube to prevent opening during heating. Do not pierce the cap.
4. **Heating block option:**
   Heat tubes for 6 minutes in a heat block at 120°C. The tubes must be placed in the block only when the prescribed temperature is reached. (*)

**OR**

**Boiling water-bath option:**
If using a boiling water bath: heat the tubes for 3 minutes at 100°C. (*)
The tubes must be placed in the block only when the prescribed temperature is reached.

5. Carefully remove the hot tubes from the heat block or the boiling water bath and place in a centrifuge. Centrifuge the tubes at 10,000 g for 10 minutes.
6. The supernatant is used for the detection of the mannan antigen. Test the supernatants using the following procedure. The test must be performed within the two hours following the serum or plasma heat-treatment.

(*) Strict compliance with the prescribed temperature and the prescribed turn-around time as well as use of the 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 the specifications using a calibrated temperature sensor 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 samples treated according to this procedure can be used for the Platelia™ Aspergillus EIA/Ag assay, since the sample treatment procedures are identical for the two tests.
- Do not store the samples (Negative Control, Positive Control and patient samples) after heat treatment.

**EIA Procedure**

Strictly comply with the proposed test protocol.
Comply with Good Laboratory Practices.
- Bring the reagents to room temperature (18-30°C) for at least 30 minutes before use.
- Use all the Calibrators, the Negative and Positive Controls with each run to validate the test results.

*Proceed as follows:*

1. Prepare a chart for the identification of the Calibrators, Negative Control, Positive Control and patient samples according to the following plan:
   - A1: Positive Control R5 (supernatant of the treated control)
   - B1: Calibrator 500 pg/ml (R4d)
   - C1: Calibrator 250 pg/ml (R4c)
   - D1: Calibrator 125 pg/ml (R4b)
   - E1: Calibrator 62.5 pg/ml (R4a)
   - F1: Calibrator 0 pg/ml (R3)
   - G1: Negative Control R0 (supernatant of the treated control)

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

3. Pipette 100 µl of supernatant of the treated patient sample and 100µl of supernatant of the treated R0 and R5 controls into the corresponding wells.
4. Then pipette 100 µl of R4d, R4c, R4b, R4a and R3 according to the plate sequence.

**NB: The distribution of the calibrator range can be controlled at this step of the procedure: the corresponding wells show colors shading from orange (R4d) to light yellow (R3).**

5. Mix the content of the vial R6 by inverting before use. If using a multichannel pipette, only take the volume necessary for a run: provide 2.5 ml for two strips of 8 wells.

6. Add 100 µl of the conjugate solution (R6) to each well.

7. Cover the microplate with an **adhesive plate sealer**, ensuring that entire surface is covered and watertight.

8. Incubate the microplate immediately in a dry microplate incubator for 90 ± 10 minutes at 37°C ± 1°C.

9. Prepare the Working Washing Solution (refer to section 8).

10. Remove the adhesive plate sealer. Aspirate the content of all wells into a biohazard waste container (containing sodium hypochlorite). Wash the microplate **5 times with a microplate washer** (using 800 µl of Working Washing Solution). After the last wash, **invert the microplate and gently tap on absorbent paper to remove the remaining liquid**.

11. Rapidly add 200 µl of Chromogen Solution (R9) to each well, **avoiding exposure to bright light**.

12. Incubate the microplate **in the dark** at +19-25°C for 30+/−5 minutes. **Do not use adhesive film during this incubation step**.

13. Add 100 µl of Stopping Solution (R10) to each well, utilizing the same order and rhythm as for addition of the Chromogen Solution. Mix well.

14. Thoroughly wipe the bottom of each plate.

15. Read the optical density of each well at 450nm (reference filter of 620 nm). Microplates must be read within 30 minutes of addition of the Stopping Solution. (the strips must always be kept away from light before reading).

16. Check concordance between the reader printout and the microplate distribution plan before transcribing the results.

**11- QUALITY CONTROL (VALIDITY CRITERIA)**

Use the calibrators and the controls on each microplate every time the test is performed.

The following criteria must be met to validate the assay:

- **Optical density value:**
  OD R4a > 0.280
  OD R0 < OD R4a
• **Ratios:**
  OD R4a / OD R3 > 1.25  
  OD R4b / OD R4a > 1.15  
  OD R4c / OD R4b > 1.15  
  OD R4d / OD R4c > 1.20  

• **Mannan concentration in the Positive Control R5:**
The R5 concentration must equal the concentration indicated on the vial ± 30%.

### 12- INTERPRETATION OF THE RESULTS

**Plotting the calibration curve**
The calibration curve is plotted using the 5 range points (calibrators): 0 pg/ml, 62.5 pg/ml, 125 pg/ml, 250 pg/ml and 500 pg/mL.  
Draw the calibration curve [OD = function (pg/ml)] by recording the ODs of the calibrators R3, R4a, R4b, R4c and R4d to the vertical axis (Y axis), then recording their respective concentration in pg/ml on the horizontal axis (X axis).  
Opt for a point to point path linking the various range points.

**Determination of the mannan concentration (pg/ml) in the test samples**
The mannan concentration reported as pg/ml can be determined from the calibration curve for each test sample.

**Interpretation of the results**
- Samples with concentrations less than 62.5 pg/mL (C < 62.5) are considered to be «negative» for mannan antigen.  
- Samples with concentrations between 62.5 and 125 pg/mL (62.5 ≤ C < 125) are considered to be «intermediate» for mannan antigen.  
- Samples with concentrations that are equal or greater than 125 pg/mL (C ≥ 125) are considered to be «positive» for mannan antigen.  
- The range points used for plotting the calibration curve do not allow any titration of concentrations above 500 pg/mL.

To determine more accurately the concentration of highly positive samples, the test must be performed again after pre-diluting to 1/5 the supernatant of the treated sample (new treatment) in the Working Washing Solution (refer to section 8).

The concentration of the highly positive samples will be calculated by multiplying the titer thus obtained by 5.

An « intermediate » result can be confirmed by collecting a new sample from the patient within a few weeks of the date of the collection of the sample which produced an intermediate result.
13- LIMITATIONS OF THE PROCEDURE

1. A negative test cannot rule out the diagnosis of invasive candidiasis because of the very low concentration and the rapid elimination of the mannan antigen during infection.

A diagnosis of invasive candidiasis can be made only if considering together the clinical, therapeutic, radiological, cytological, direct mycological and serological data, with any single criteria being interpreted with caution.

2. A negative test for mannan antigen must also be interpreted in conjunction with the results of anti-mannan antibody test: even in case of invasive candidiasis, the mannan antigen is more difficult to detect in patients tested positive for anti-mannan antibodies (refer to section 15-Performances).

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

4. The Platelia™ Candida Ag Plus procedure and the interpretation of the 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 reagents as instructed in the procedure could result in a falsely negative result. Repeat testing of additional samples should be considered where there is clinical suspicion of invasive candidiasis or procedural error.

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

7. The performance characteristics of Platelia™ Candida Ag Plus have not been evaluated with neonatal or pediatric serum or plasma samples.

8. The Performance characteristics of Platelia™ Candida Ag Plus have not been established for manual reading and/or visual result determination.

9. There has been report of cross reaction in patients receiving an infusion with certain batches of hydrox yethylstarch plasma expanders (such as Hesteril® 6%), used in the treatment of circulatory failures: hypovolaemia, hemorrhagic shock and septic shock.
10. Falsely positive results have been observed with samples containing a concentration of human gammaglobulins $\geq 60$ g/l and also with some samples that have been tested and found to be reactive for anti-toxoplasma antibodies.

14- EXPECTED VALUES
The prevalence of mannan Candida antigen measured using the Platelia™ Candida Ag Plus test was evaluated on a panel of 613 samples from 51 Dutch patients (Site 1- The Netherlands) hospitalized for treatment of cancer (oncohematological pathology in 49 patients and non-hematological cancer in 2 patients) by intensive chemotherapy.

Of 613 samples, 88 proved positive and 51 intermediate for a prevalence of $88/613 = 14.4 \%$ [IC 95%: 11.7-17.4%] considering the intermediate results as negative and $139/613 = 22.7 \%$ [IC 95%: 19.4-26.2%] considering the intermediate results as positive. In terms of patients, of 51 tested, 23 had at least one positive sample and 14 had at least one intermediate sample and no positives, for a prevalence of $23/51 = 45.1 \%$ [IC 95%: 31.1-59.7%] considering the intermediate results as negative and $37/51 = 72.6 \%$ [IC 95%: 58.3-84.1%] considering the intermediate results as positive.

Of these 51 patients, 30 (388 samples) did not present documented invasive candidiasis. Amongst the latter, 20 were colonized by yeasts (12 by Candida albicans, 7 by Candida albicans associated with another species of Candida and 1 colonized by at least one Candida non-albicans species). Four of these patients presented clinical symptoms, and microbiological evidence of a superficial infection to Candida. Severe mucosal barrier injury was present in 24 of these patients. Of the corresponding 388 samples, 41 were positive and 43 intermediate, for a prevalence of $41/388 = 10.6 \%$ [IC 95%: 7.7-14.1%] considering the intermediate results as negative and $84/388 = 21.7 \%$ [IC 95%: 17.7-26.1%] considering the intermediate results as positive. In terms of patients, of 30 tested, 10 showed at least one positive sample and 11 presented at least one intermediate sample and no positives, for a prevalence of $10/30 = 33.3 \%$ [IC 95%: 17.3-52.8%] considering the intermediate results as negative and $21/30 = 70.0 \%$ [IC 95%: 50.6-85.3%] considering the intermediate results as positive.

15- PERFORMANCE CHARACTERISTICS
A. Reproducibility studies
• Within-run precision (repeatability):
In order to evaluate intra-assay repeatability, the five samples (two negative and three positive) were tested 32 times during the same run. The concentration in pg/ml was determined for each sample.
The mean concentration, the Standard Deviation (SD) and Coefficient of Variation (%CV) for each sample are listed in the table below:

**Within-run precision (repeatability)**

<table>
<thead>
<tr>
<th></th>
<th>N=32</th>
<th>Negative Sample</th>
<th>High negative sample</th>
<th>Low positive sample</th>
<th>Mean positive sample</th>
<th>High positive sample</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean concentration (pg/ml)</strong></td>
<td></td>
<td>25.1</td>
<td>54.8</td>
<td>63.7</td>
<td>154.1</td>
<td>261.8</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td></td>
<td>4.11</td>
<td>5.20</td>
<td>4.27</td>
<td>8.01</td>
<td>16.93</td>
</tr>
<tr>
<td><strong>CV %</strong></td>
<td></td>
<td>16.3%</td>
<td>9.5%</td>
<td>6.7%</td>
<td>5.2%</td>
<td>6.5%</td>
</tr>
</tbody>
</table>

- Between-run precision (reproducibility):
In order to evaluate inter-assay reproducibility, the five samples (two negative and three positive) were each tested in duplicate, in two series per day, over a 20 day period. The concentration in pg/ml was determined for each sample. The mean concentration, the Standard Deviation (SD) and Coefficient of Variation (%CV) for each sample are listed in the table below:

**Between-run precision (reproducibility)**

<table>
<thead>
<tr>
<th></th>
<th>N=80</th>
<th>Negative Sample</th>
<th>High negative sample</th>
<th>Low positive sample</th>
<th>Mean positive sample</th>
<th>High positive sample</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean concentration (pg/ml)</strong></td>
<td></td>
<td>24.1</td>
<td>55.0</td>
<td>70.0</td>
<td>161.6</td>
<td>264.9</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td></td>
<td>6.99</td>
<td>11.47</td>
<td>14.50</td>
<td>30.07</td>
<td>38.62</td>
</tr>
<tr>
<td><strong>CV %</strong></td>
<td></td>
<td>29.0%</td>
<td>20.8%</td>
<td>20.7%</td>
<td>18.6%</td>
<td>14.6%</td>
</tr>
</tbody>
</table>
B. Cross Reactivity

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Number of samples tested</th>
<th>Number of positives</th>
<th>Number of intermediates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus</td>
<td>10</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Anti-ds-DNA antibodies</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ANAs Positive</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>myeloma IgG and IgM</td>
<td>20</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Anti-Toxoplasma IgG</td>
<td>10</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Human anti-mouse antibodies</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rheumatoid Factor</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

C. Linearity
The range of linearity of the Platelia™ Candida Ag Plus test has been established as between 20 and 470 pg/ml from studies of dilutions made of 4 positive samples.

D. Clinical studies
The performance characteristics of the Platelia™ Candida Ag Plus kit have been evaluated at 3 sites with a total of 852 samples from 505 patients.

SENSITIVITY
The sensitivity of the Platelia™ Candida Ag Plus kit was determined on a panel of 436 samples from 89 patients hospitalized at 2 sites (in The Netherlands, in France). The distribution of the samples is as follows:

- Site 1 (The Netherlands): 225 samples from 21 Dutch patients admitted to an oncohematological ward for treatment of malignant hemopathy (19 cases) or a non hematological cancer (2 cases) by intensive chemotherapy, followed, if appropriate, by an hematopoietic stem cell transplantation. All these patients presented an invasive candidiasis microbiologically proved on the basis of at least one culture positive to Candida (blood culture or culture of normally sterile tissue sample). 17
- Site 2 (France): 211 samples from 68 French patients hospitalized in various intensive care or oncohematology wards with invasive candidiasis microbiologically proved on the basis of at least one blood culture positive to Candida spp.
NB: The sample panels used in these two studies are from patients whose blood samples were collected between 1999 and 2007. The relative stability of the mannan antigen and the anti-mannan antibodies in these samples during the freezing period, and also after each freezing and thawing cycle, make the results dependent on the storage conditions of the tested panel. The sensitivity values of the test determined conducting retrospective clinical studies can thus be lower than those obtained conducting prospective clinical studies, using samples collected very recently.

Results from Site 1

The 21 Dutch patients were admitted to hospital for treatment of malignant hemopathy (acute myeloid leukaemia, acute lymphatic leukaemia, chronic lymphatic leukaemia, myeloma, myelodysplastic syndrome, aplastic anaemia, or non-Hodgkin lymphoma) or a non haematological cancer by intensive chemotherapy followed, if appropriate, by an hematopoietic stem cell transplantation. These 21 patients developed an invasive candidiasis microbiologically proved on the basis of at least one culture positive to *Candida spp.* (blood culture or culture of normally sterile tissue sample). The status of these patients for the presence of the mannan *Candida* antigen, detected by the Platelia™ *Candida* Ag Plus test, is considered as positive if at least one of the patient samples is positive. In the absence of a positive sample, the status is considered as intermediate if at least one of the patient samples is intermediate and as negative when all the patient samples are negative. 17

Alongside the Platelia™ *Candida* Ag Plus test, the same samples were tested for the presence of the anti-mannan antibodies with the Platelia™ *Candida* Ab Plus kit 9. The status of the patient for the presence of the mannan *Candida* antigen or anti-mannan *Candida* antibodies is considered as positive if at least one of the two tests is positive. In the absence of a positive test, the status is considered as intermediate if at least one of the two tests is intermediate and as negative when the two tests are negative.

<table>
<thead>
<tr>
<th>Categories of patients</th>
<th>Results in Platelia™ <em>Candida</em> Ag Plus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of patients (number of samples)</td>
</tr>
<tr>
<td>21 patients (225 samples) presenting at least one culture positive to <em>Candida spp.</em></td>
<td>positive</td>
</tr>
<tr>
<td></td>
<td>13 (47)</td>
</tr>
</tbody>
</table>
Results from Site 2

The 68 French patients were admitted to various intensive care (surgery, transplants, burns, trauma, pneumology, etc...) or oncohematology (malignant hemopathy) wards. They all developed an invasive candidiasis microbiologically proved on the basis of at least one blood culture positive to *Candida* (*albicans*, *parapsilosis*, *norvegensis*, *glabrata*, *krusei* or *tropicalis*) \(^6,7,14\) in the days prior to or following the studied samples. The blood samples were collected 6 days (in average) after the first blood culture positive to *Candida* (minimum 61 days before, maximum 67 days after). The status of these patients for the presence of the mannan *Candida* antigen is considered as positive if at least one of the patient samples is positive. In the absence of a positive sample, the status is considered as intermediate if at least one of the patient samples is intermediate and as negative when all the samples are negative.

<table>
<thead>
<tr>
<th>Categories of patients</th>
<th>Combined results in Plateelia™ <em>Candida</em> Ag Plus and Plateelia™ <em>Candida</em> Ab Plus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of patients (number of samples)</td>
</tr>
<tr>
<td></td>
<td>positive</td>
</tr>
<tr>
<td>21 patients (225 samples) presenting at least one culture positive to <em>Candida</em> spp.</td>
<td>15 (98)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Categories of patients</th>
<th>Results in Plateelia™ <em>Candida</em> Ag Plus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of patients (number of samples)</td>
</tr>
<tr>
<td></td>
<td>positive</td>
</tr>
<tr>
<td>68 patients (211 samples) presenting at least one blood culture positive to <em>Candida</em> spp.</td>
<td>35 (86)</td>
</tr>
<tr>
<td>- including 34 to <em>C. albicans</em> (113 samples)</td>
<td>22 (55)</td>
</tr>
<tr>
<td>- including 15 to <em>C. parapsilosis</em> (37 samples)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>- including 12 to <em>C. glabrata</em> (32 samples)</td>
<td>7 (13)</td>
</tr>
<tr>
<td>- including 4 to <em>C. tropicalis</em> (19 samples)</td>
<td>4 (15)</td>
</tr>
<tr>
<td>- including 2 to <em>C. krusei</em> (8 samples)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>- including 1 to <em>C. norvegensis</em> (2 samples)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

*: the confidence interval of 95% was not calculated due to insufficient sample size.
Alongside the Platelia™ *Candida* Ag Plus test, the same samples were tested for the presence of the anti-mannan antibodies with the Platelia™ *Candida* Ab Plus kit. The status of the patient for the presence of the mannan *Candida* antigen or anti-mannan *Candida* antibodies is considered as positive if at least one of the two tests is positive. In the absence of a positive test, the status is considered as intermediate if at least one of the two tests is intermediate and as negative when the two tests are negative.

<table>
<thead>
<tr>
<th>Categories of patients</th>
<th>Combined results in Platelia™ <em>Candida</em> Ag Plus and Platelia™ <em>Candida</em> Ab Plus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of patients (number of samples)</td>
</tr>
<tr>
<td></td>
<td>positive</td>
</tr>
<tr>
<td>68 patients (211 samples) presenting at least one blood culture positive to <em>Candida</em> spp.</td>
<td>48 (122)</td>
</tr>
<tr>
<td>- including 34 to <em>C. albicans</em> (113 samples)</td>
<td>28 (74)</td>
</tr>
<tr>
<td>- including 15 to <em>C. parapsilosis</em> (37 samples)</td>
<td>5 (7)</td>
</tr>
<tr>
<td>- including 12 to <em>C. glabrata</em> (32 samples)</td>
<td>11 (24)</td>
</tr>
<tr>
<td>- including 4 to <em>C. tropicalis</em> (19 samples)</td>
<td>4 (17)</td>
</tr>
<tr>
<td>- including 2 to <em>C. krusei</em> (8 samples)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>- including 1 to <em>C. norvegensis</em> (2 samples)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

* the confidence interval of 95% was not calculated due to insufficient sample size.

**SPECIFICITY**

Specificity was determined on a panel of 416 samples from 2 French sites, the distribution of the samples being as follows:

- Site 1: 200 samples from 200 French female patients monitored for toxoplasmosis serology during their pregnancy and with no clinical symptom of *Candida* infection.
- Site 2: 219 samples from 216 French blood donor patients.
16- MANUFACTURER’S QUALITY CONTROL

All manufactured reagents are prepared according to our Quality System, starting from reception of raw material to commercialization of the final product. Each lot is submitted to quality control assessments and is released to the market only after conforming to pre-defined acceptance criteria. The records related to production and controls of each single lot are kept within Bio-Rad.

17- BIBLIOGRAPHY


**R43:**
- May cause sensitisation by skin contact.

**S24-37-60:**
- Avoid contact with skin. Wear suitable gloves. This material and its container must be disposed of as hazardous waste.
(LV) • Nepielaut noklūšanu uz ādas. Strādāt aizsargcimdos. Apglabāt šo produktu un tās iepakojumu kā bistamos atkritumus.

(MT) • Evita l-kuntatt mal-gilda. Ilbes ingwanti adatt. Dan il-materjal u l-kontenitur tieghu ġhandhom jintremew ma’skart perikoluż.

(NL) • Aanraking met de huid vermijden. Draag geschikte handschoenen. Deze stof en de verpakking als gevaarlijk afval afvoeren.

(SI) • Preprečiti stik s kožo. Nositi primerne zaščitne rokavice. Snov/pripravek in embalažo odstraniti kot nevarni odpadek.

(FI) • Varottava kemikaalin joutumista iholle. Käytettävä sopivia suojakäsineitä. Tämä aine ja sen pakkaus on käsiteltävä ongelmajätteenä.