PLATELIA™ *ASPERGILLUS* EIA

96 TESTS 62796

THE PLATELIA™ *ASPERGILLUS* EIA IS AN IMMUNOENZYMATIC SANDWICH MICROPLATE ASSAY FOR THE DETECTION OF *ASPERGILLUS* GALACTOMANNAN ANTIGEN IN SERUM

IVD

BIO-RAD

1- INTENDED USE

The Platelia™ *Aspergillus* EIA is an immunoenzymatic sandwich microplate assay for the detection of *Aspergillus* galactomannan antigen in serum samples.

2- INDICATIONS FOR USE

The Platelia™ *Aspergillus* EIA is a test which, when used in conjunction with other diagnostic procedures such as microbiological culture, histological examination of biopsy samples and radiographic evidence can be used as an aid in the diagnosis of Invasive Aspergillosis.

3- SUMMARY AND EXPLANATION

Aspergillus infections usually occur following inhalation of *Aspergillus* spores which are present in the environment. Invasive forms, which have been on the increase for the past 10 years, constitute the most serious infections. They mainly occur in neutropenic patients (following anti-cancer treatment) and in patients treated with immunosuppressants (organ transplantations, particularly bone marrow transplantation) and corticosteroids [7].

Aspergillus is rarely isolated from blood culture. The diagnosis is often based on nonspecific diagnostic or radiological evidence (clinical symptoms, CT scan, chest x-ray, etc.)

At the present time, the test for soluble galactomannan antigen in serum appears to be a serological method able to aid in the diagnosis of Invasive Aspergillosis [6, 9, 14, 34, 39].

4- PRINCIPLE OF THE PROCEDURE [27]

The Platelia™ *Aspergillus* EIA is a one-stage immunoenzymatic sandwich microplate assay which detects galactomannan in human serum. The assay uses the rat EBA-2 monoclonal antibodies, which are directed against *Aspergillus galactomannan*, and have been characterized in previous studies [16, 28]. The monoclonal antibodies are used, (1) to coat the wells of the microplate and bind the antigen, and (2) to detect the antigen bound to the sensitized microplate (conjugate reagent: peroxidase-linked monoclonal antibodies). 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 [15]. The treated serum samples and conjugate are added to the wells coated with monoclonal antibodies, and incubated. A monoclonal antibody - galactomannan - monoclonal antibody / peroxidase complex is formed in the presence of galactomannan 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 and controls is determined with a spectrophotometer set at 450 and 620 nm wavelength.

5- REAGENTS

Platelia™ *Aspergillus* EIA: product No. 62796 (96 Tests)

Store the kit at 2-8°C. Bring all reagents to room temperature (18-25°C) before use. Return all reagents, except controls, to 2-8°C immediately after use. After reconstitution, unused Negative Control, Cut-off Control, and Positive Control must be frozen at -20°C. Return unused strips/plates to pouch and reseal. Do not remove dessicant. 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 9 batches.

Component		Contents	Quantity
R1	**Microwell Strip Plate**	**Microplate:** - 96 wells (12 strips of 8 wells each) coated with anti-galactomannan monoclonal antibodies	1 Plate / 12 x 8 Wells
R2	**Concentrated Washing Solution**	**Concentrated Washing Solution (10X):** - Tris NaCl buffer - 1% Tween® 20 - 0.01% thimerosal	1 x 100 mL
R3	**Negative Control Serum**	**Negative Control Serum:** - Freeze-dried human serum negative for galactomannan - Negative for anti-HIV-1, anti-HIV-2, anti-HCV antibodies and HBs antigen	3 x qs* 1 mL
R4	**Cut-off Control Serum**	**Cut-off Control Serum:** - Freeze-dried human serum containing galactomannan - Negative for anti-HIV-1, anti-HIV-2, anti-HCV antibodies and HBs antigen	3 x qs* 1mL
R5	**Positive Control Serum**	**Positive Control Serum:** - Freeze-dried human serum containing galactomannan - Negative for anti-HIV-1, anti-HIV-2, anti-HCV antibodies and HBs antigen	3 x qs* 1 mL
R6	**Conjugate**	**Conjugate (ready to use):** - Anti-galactomannan monoclonal antibody / peroxidase labeled - Preservative: 0.01% thimerosal	1 x 8 mL
R7	**Serum Treatment Solution**	**Serum Treatment Solution (ready to use):** - EDTA acid solution	1 x 10.5 mL
R8	**TMB Substrate Buffer**	**TMB Substrate Buffer (ready to use):** - Citric acid and sodium acetate - 0.009% hydrogen peroxide - 4% dimethylsulfoxide (DMSO)	1 x 60 mL
R9	**Chromogen: TMB Solution**	**Chromogen TMB Solution (concentrated):** - 90% dimethylsulfoxide (DMSO) solution containing 0.6% tetramethylbenzidine (TMB)♦	1 x 1 mL
R10	**Stopping Solution**	**Stopping Solution (ready to use):** - 1.5 N sulphuric acid (H_2SO_4)	1 x 12 mL
	Plate sealers	- Adhesive sheets for microplates	1 x 8 sheets

•***Note***: TMB (tetramethylbenzidine) is a non-carcinogenic and non-mutagenic chromogen for peroxidase.

****Note***: qs: quantity sufficient.

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 Positive Control, Cut-off Control, and Negative Control are manufactured from human serum that has been tested and found to be non-reactive for HBsAg and antibodies to HIV-1, HIV-2 and HCV with CE marked tests. However, all reagents should be handled as though capable of transmitting infection. 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 patient samples with the requisite Good Laboratory Practices. 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 are not limited to) a solution of 10% bleach (0.5% solution of sodium hypochlorite), 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.
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 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 local, regional and national regulations.
12. **CAUTION**: The following is a list of potential chemical hazards contained in some kit components (refer to section 5- REAGENTS):


C-Corrosive

The 1.5 N Sulfuric Acid (7.2% H_2SO_4) Stopping Solution is Corrosive, capable of causing burns to the eyes and skin; may be harmful if swallowed, or in contact with skin; can cause serious eye damage, including permanent impairment of vision or blindness.

Keep away from strong bases and reducing agents.

R34-41 Causes burns. Risk of serious damage to eyes.

S24/25-26-30-36/37/39-60. Avoid contact with skin and eyes. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Never add water to this product. Wear suitable protective clothing, gloves and eye/face protection. This material and its container must be disposed of as hazardous waste.

Waste from this material is considered hazardous acidic waste, however if permitted by local, regional, and national regulations, it might be neutralized to pH 6-9 for non-hazardous disposal, if trained and equipped to do so.

0.01% Thimerosal (Merthiolate Sodium), an Organo-Mercury biocidal preservative that targets the central nervous system (CNS), is a reproductive toxicant and significant sensitizer; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals; there are ample cases of sensitization resulting from exposure to dilute Thimerosal solutions. Avoid release to the environment, danger of cumulative effects. Spent mercury-containing solutions with a concentration greater than 0.2 ppm must be disposed of as US Federal RCRA hazardous waste (D009), however, dispose of all wastes in accordance with local, regional, and national regulations. (Note: mercury (Hg) makes up 49.55% of the Thimerosal molecule, thus a component with 0.01% Thimerosal contains ~0.005% (~50 ppm) mercury w/v).

In case of contact with skin, wash immediately with plenty of water. Warning: Thimerosal is known to the State of California to cause reproductive toxicity.

13. 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, exercising 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. Use clean, dust-free materials (tubes, tips, containers, etc.) to minimize the possibility of contamination with Aspergillus spores from the environment. Because galactomannan is heat-stable, sterilization of material used does not guarantee the absence of contaminating antigen. Pyrogen-free materials are optimal, but standard material can be used with adequate precautions.
16. Limit exposure of solutions (sera, Serum Treatment Solution, Conjugate) or open containers (plates, tubes, pipettes) to the air.
17. Do not pour any unused Conjugate back into the original container.
18. 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 dessicant.

Working Washing Solution (R2)

Prepare Working Washing Solution as needed by adding one part Concentrated Washing Solution to 9 parts sterile deionized or distilled water. The Working Washing Solution can be stored for 14 days at 2-8°C. Prepare a sufficient amount of Working Washing Solution to complete the run (80 mL for one strip: 8 mL R2 + 72 mL distilled water).

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.

Negative Control Serum (R3)

Reconstitute the contents of one bottle of control with 1000 µL (1 mL) of sterile, purified water (preferably pyrogen-free water). The sera must be rehydrated just before performing the test. Mix thoroughly after allowing 2-3 minutes for rehydration of the serum. Aliquot 300 µL into each of 3 polypropylene microcentrifuge tubes. Immediately freeze at -20°C any remaining polypropylene centrifuge tubes that will not be used after rehydration.

Note: Control sera that have been previously rehydrated and immediately frozen at -20°C may be thawed and used without further rehydration. Frozen rehydrated controls may be stored at -20°C for up to five weeks. Handle the control sera in the same manner as patient specimens (300 µL of serum + 100 µL of Serum Treatment Solution, etc...).

Cut-off Control Serum (R4) and Positive Control Serum (R5)

Prepare as described above for Negative Control Serum.

Substrate-Chromogen Reaction Solution (R8 + R9)

Prepare 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.

Conjugate (R6), Serum Treatment Solution (R7) and Stopping Solution (R10)

After opening, 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. Unopened samples can be stored at 2-8°C for up to 5 days prior to testing. After initial opening, samples may be stored at 2-8°C for 48 hours prior to testing. For longer storage, store the serum at – 70°C.

Serum samples can be subjected to a maximum of 4 freezing / thawing cycles. Previously frozen specimens should be thoroughly mixed after thawing prior to testing.

The results are not affected by samples containing 20 mg/L of bilirubin, lipemic samples containing the equivalent of 2 g/L of triolein (triglyceride) or hemolysed samples containing 165 mg/L of hemoglobin. Interferences related to excess albumin have not been tested.

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. Sterile purified water for reconstitution of control sera.
3. Absorbent paper.
4. Disposable gloves.

5. Protective glasses.
6. Sodium hypochlorite (bleach) and sodium bicarbonate.
7. Pipettes or multipipettes, adjustable or fixed, to measure and dispense 50 µL, 100 µL, 300 µL, and 1000 µL.
8. 1.5 mL polypropylene microcentrifuge tubes with airtight stoppers, able to support heating to 120°C (heat block) or 100°C (boiling water bath).
   a. Screw cap tubes: 1.5 mL Conical Tubes, Bio-Rad Cat. # 224-0100 or equivalent.
   b. Snap cap tubes: EZ Micro Test Tubes, 1.5 mL, Bio-Rad Cat. # 223-9480 or equivalent.
9. 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.
10. Laboratory bench centrifuge for polypropylene 1.5 mL tubes capable of obtaining 10,000g.
11. Floating micro-centrifuge rack.
12. Vortex agitator.
13. Heat block. The following heat block models are recommended:
    a. 1 block model: Grant Cat. # QBD1 (VWR Cat. # 460-0074)
    b. 2 block model: Grant Cat. # QBD2 (VWR Cat. # 460-0076)
14. Block for heat block: both heat blocks must be used with Grant block Cat. # QB-E1 (VWR Cat. # 460-8517)
15. Boiling water bath at 100°C
16. Microplate incubator at 37 ± 1°C.
17. Semi-automated or automated microplate washer.
18. Microplate reader equipped with 450 nm and 620 nm filters.

Procedural Comments

Negative, Positive, and Cut-off Controls must be tested on each run to validate the test results.

Treatment of the sera

All control sera: negative (R3), cut-off (R4) and positive (R5) must be processed at the same time as serum samples:

1. Pipette 300 µL of each test serum and control 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, for snap cap tubes: use a cap lock. 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. (*)
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.
6. The supernatant is used for detection of the galactomannan antigen.
7. Test the supernatants using the following procedure. After preparation, the supernatant may be removed and stored at 2-8°C for up to 48 hours prior to testing. 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, please 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.

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, and Negative, Positive, and Cut-off Controls.
3. Prepare a chart for identification of test sera and controls in the microplate. Use one well for the Negative Control Serum (R3), two wells for the Cut-off Control Serum (R4), and one well for the 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 dessicant, and reseal the pouch.
5. Mix the contents of the Conjugate bottle (R6) by inverting before use. Add 50 µL of Conjugate (R6) 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 to 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 nm). Microplates must be read within 30 minutes of addition of Stopping Solution.

11- QUALITY CONTROL (VALIDITY CRITERIA)

Cut-off Control: The O.D. of each Cut-off Control Serum must be
≥ 0.300 and ≤ 0.800.

Positive Control: The index of the Positive Control Serum must be greater than 2.00.

$$I = \frac{\text{OD Positive Control (R5)}}{\text{Mean Cut-off Control OD}} > 2.00$$

Negative Control: The index of the Negative Control Serum must be less than 0.40.

$$I = \frac{\text{OD Negative Control (R3)}}{\text{Mean Cut-off Control OD}} < 0.40$$

Failure of any of the controls to meet the validity criteria described above renders the assay invalid, and patient specimen results should not be reported. The operator may decide to repeat the assay, after reviewing the procedure, or may contact the manufacturer for assistance. If a repeat assay is performed, then a new aliquot of the same sample should be used in the repeat assay.

Example Calculation:

Sample	Absorbance (OD)
Negative Control (R3) OD	0.117
Cut-off Control (R4) OD	0.596 0.576
Positive Control (R5) OD	2.602

Calculations

Mean Cut-off Control Value

To calculate the mean Cut-off Control (R4) OD, add the OD values for each Cut-off Control replicate together and divide the result by 2:
(0.596 + 0.576) ÷ 2 = 0.586

Negative Control Index

To calculate the index of the Negative Control, divide the OD of the Negative Control by the mean Cut-off Control OD:

$$I = \frac{0.117}{0.586} = 0.20$$

Positive Control Index

To calculate the index of the Positive Control, divide the OD of the Positive Control by the mean Cut-off Control OD:

$$I = \frac{2.602}{0.586} = 4.44$$

Validity

In the above example:

- Each Cut-off Control OD is ≥ 0.300 and ≤ 0.800, indicating that the Cut-off Control is valid.
- The index of the Negative Control is < 0.40, indicating that the Negative Control is valid.
- The index of the Positive Control is > 2.00, indicating that the Positive Control is valid.
  The test run in this example is considered to be valid since the results meet the validity criteria for each control.

12- INTERPRETATION OF RESULTS

The presence or absence of galactomannan antigen in the test sample is determined by calculation of an index for each patient specimen. The Index (I), is the OD value of the specimen divided by the mean optical density of the wells containing Cut-off Control Serum.

Calculation of the mean Cut-off Control optical density:

Add the optical densities of the two wells containing Cut-off Control Serum (R4) and divide the total by 2.

Calculation of an index (I) for each test serum:

Calculate the following ratio for each test serum:

$$I = \frac{\text{OD sample}}{\text{Mean Cut-off Control OD}}$$

Sera with an index < 0.50 are considered to be negative for galactomannan antigen.

Note: A negative result may indicate that the patient's result is below the detectable level of the assay.

Negative results do not rule out the diagnosis of Invasive Aspergillosis. Repeat testing is recommended if the result is negative, but the disease is suspected.

Sera with an index ≥ 0.50 are considered to be positive for galactomannan antigen.

For all positive patients, it is recommended that a new aliquot of the same sample be repeated as well as collection of a new sample from the patient for follow-up testing.

Note: An absorbance value of less than 0.000 may indicate a procedural or instrument error which should be evaluated. That result is invalid and the specimen must be re-run.

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

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

Example Calculation:

Sample	Absorbance (OD)
Negative Control (R3) OD	0.117
Cut-off Control (R4) OD	0.596 0.576
Positive Control (R5) OD	2.602
Patient Sample #1	0.134
Patient Sample #2	0.436
Patient Sample #3	1.196

Calculations

Refer to the Quality Control (Validity Criteria) section for an example of calculations to determine the validity of the assay controls.

Mean Cut-off Control Value

To calculate the mean Cut-off Control (R4) OD, add the OD values for each Cut-off Control replicate together and divide the result by 2:

$(0.596 + 0.576) \div 2 = 0.586$

Patient Sample #1

To calculate the index of Patient Sample #1, divide the OD of Patient Sample #1 by the mean Cut-off Control OD:

$$I = \frac{0.134}{0.586} = 0.23$$

In this example, Patient Sample #1 is negative, since the Index of 0.23 is < 0.50.

Patient Sample #2

To calculate the index of Patient Sample #2, divide the OD of Patient Sample #2 by the mean Cut-off Control OD:

$$I = \frac{0.436}{0.586} = 0.74$$

In this example, Patient Sample #2 is positive, since the Index of 0.74 is ≥ 0.50.

Patient Sample #3

To calculate the index of Patient Sample #3, divide the OD of Patient Sample #3 by the mean Cut-off Control OD:

$$I = \frac{1.196}{0.586} = 2.04$$

In this example, Patient Sample #3 is positive, since the Index of 2.04 is ≥ 0.50.

13- LIMITATIONS OF THE PROCEDURE

1. **A negative test cannot rule out the diagnosis of Invasive Aspergillosis. Patients at risk for Invasive Aspergillosis should be tested twice a week**.
2. The Platelia™ *Aspergillus* EIA Procedure and the Interpretation of Results must be followed when testing samples for the presence of galactomannan 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.
3. 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 Aspergillosis or procedural error**.
4. 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.
5. The performance of the Platelia™ *Aspergillus* EIA has not been evaluated with neonatal or pediatric serum samples.
6. The Platelia™ *Aspergillus* EIA may exhibit reduced detection of galactomannan in patients with chronic granulomatous disease (CGD) and Job's syndrome [36, 37].
7. The concomitant use of mold-active anti-fungal therapy in some patients with Invasive Aspergillosis may result in reduced sensitivity with the Platelia™ *Aspergillus* EIA [20].
8. The Platelia™ *Aspergillus* EIA has not been evaluated for use with plasma or other sample types such as urine, BAL, or CSF.
9. The performance of the Platelia™ *Aspergillus* EIA has not been established for manual reading and/or visual result determination.
10. Other genera of fungi such as *Penicillium, Alternaria, Paecilomyces, Geotrichum* and *Histoplasma* have shown reactivity with rat, EBA-2 monoclonal antibodies used in the assay for the detection of *Aspergillus galactomannan*. Histoplasmosis should be considered in endemic areas including parts of the United States [23, 32, 38].
11. Positive reactions with no clinical signs:

    Considering the early detection of the galactomannan antigen detection in serum even before apparition of clinical and/or radiological features, positive reactions without clinical signs are usually observed: they correspond to "true positive" tests in patients for whom Proven or Probable Invasive Aspergillosis diagnosis is established later on.

    However, in some particular cases, some factors should be taken into account when interpreting the test:

    a. Positive reactions with no clinical signs have been reported, especially in young children [26]. Although some of these cases could be related to real circulation of *Aspergillus* antigens [5], most cases can be considered to be false-positives.
    b. Galactofuranose has been demonstrated in various foods, particularly cereals, cereal products and cream desserts [1, 17]. Unlike human milk, humanized milks frequently contain high concentrations of galactomannan [10]. The dietary factor must therefore be taken into account in interpretation of the course of antigenemia in young children, and more generally in all patients with an altered intestinal barrier [4, 10]. Any case of positive antigenemia not accompanied by clinical signs should be interpreted even more cautiously in this population of patients [17].
    c. There have been reports of positive galactomannan test results in patients receiving piperacillin / tazobactam. There have also been reports of certain lots or batches of piperacillin / tazobactam that have been found to be positive for galactomannan antigen. Therefore, positive test results in patients receiving piperacillin / tazobactam should be interpreted cautiously and confirmed by other diagnostic methods. Detection of galactomannan has also been reported in some batches of amoxicillin associated with clavulanic acid parenteral preparations. Therefore, semi-synthetic ß-lactam treatments should be taken into account when interpreting the test [1, 21].

Nevertheless, as Platelia™ *Aspergillus* EIA can detect galactomannan antigen well before clinical or radiological signs appear, the occurrence of Invasive Aspergillosis cannot be ruled out. Therefore, patients treated with piperacillin / tazobactam with positive test results should be followed carefully.

d. Positive reactions in the absence of clinical signs may be observed in patients receiving products containing galactomannan, either parenterally or orally (in the presence of an alteration of the intestinal barrier).

The presence of galactomannan in these products can often be explained by the use of a fermentation process based on fungal microorganisms.

A positive result will not be observed in a patient, however, unless the serum concentration of exogenous galactomannan reaches or exceeds the test's detection threshold.

Thus, if there is a suspicious positive result in the absence of other evocative signs, we recommend investigating the products that the patient is taking and notably their production processes and the origin of the raw materials used [11, 24, 31].

14- EXPECTED VALUES

The expected prevalence of Invasive Aspergillosis varies with the patient population; rates from 5-20% have been reported [7, 13].

A clinical study was conducted on a total of 4873 serum samples from 239 episodes (203 patients) with hematologic malignancy or hematopoetic stem cell transplant diagnosed with and without Invasive Aspergillosis, at two testing centers in Europe to determine the performance characteristics of the Platelia™ *Aspergillus* EIA.

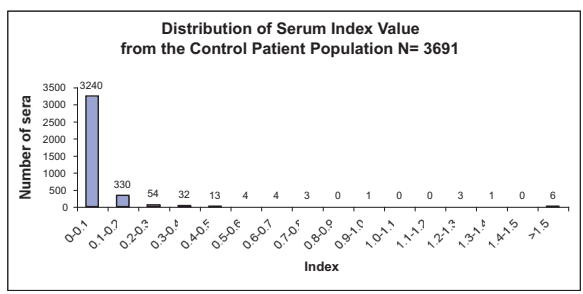
As a patient could be included in the study on more than one occasion, analysis was performed according to per treatment episodes.

An episode is considered to be the time period surrounding a clinical event (e.g. Transplant, GVHD etc.) As a result, more than one episode may have been observed for a single patient.

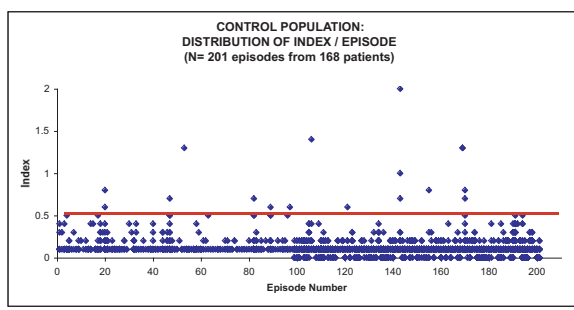
The average prevalence rate for this study was 16% (38/239). The distribution of index values for these populations is represented in the following charts.

Patients diagnosed without Invasive Aspergillosis (control population)

A total of 3691 serum samples obtained from 201 episodes (168 patients) at two testing centers in Europe were tested with the Platelia™ *Aspergillus* EIA test. The distribution of index values is shown in the following chart.

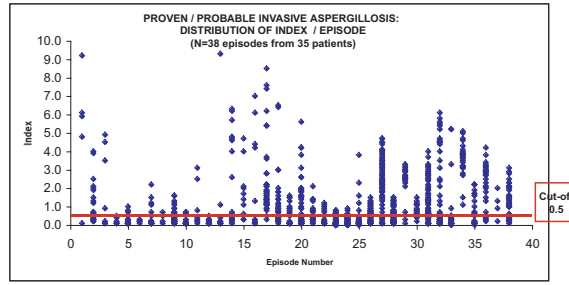


This scatter plot depicts galactomannan assay results for the 3691 serum samples from 201 episodes (168 control patients) in this study (patients undergoing immunosuppressive therapy for HSCT, or to treat hematological malignancy).



Patients diagnosed with Invasive Aspergillosis

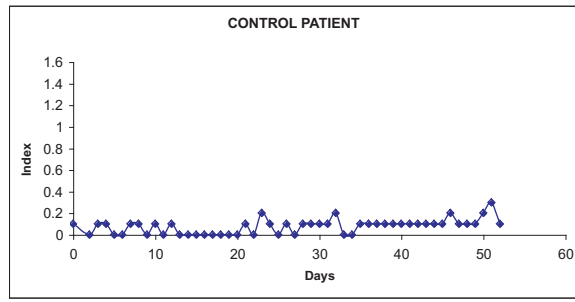
This scatter plot depicts galactomannan assay results for the 1182 serum samples from 38 episodes (35 patients) in this study diagnosed with Proven or Probable Invasive Aspergillosis as defined by EORTC/NIAID definitions. Not every serum sample from each patient is expected to be positive.



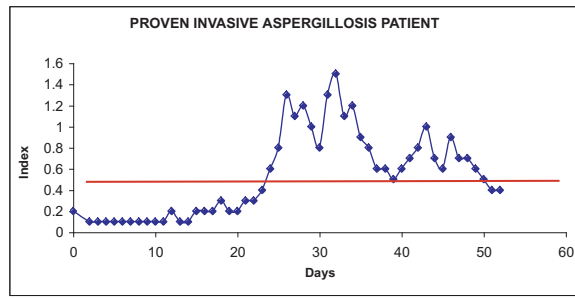
The expected prevalence of Invasive Aspergillosis varies with the patient population; rates from 5-20% have been reported [7, 13]. The prevalence rate for this study was 16%.

The following graphs represent examples of a patient without clinical signs or symptoms of Invasive Aspergillosis (negative for *Aspergillus*) and a patient with Proven Invasive Aspergillosis (positive for *Aspergillus*) respectively.

Negative patient:



Positive patient:



15. SPECIFIC PERFORMANCE CHARACTERISTICS

A. Reproducibility Studies

Inter-assay and Intra-assay variability for the Platelia™ *Aspergillus* EIA were determined in a study using a panel of 6 pooled patient serum samples (one negative, one low positive, two positive, and two high positive) obtained from three clinical trial sites in North America.

Each of the 6 panel members was tested in triplicate (x3) on 3 different days, on one lot, at two sites (total number of replicates at each site = 9). Each of the 6 panel members was tested in duplicate (x2) on three different days, on 1 lot, at a third site (total number of replicates at the third site = 6). One (1) operator performed all precision testing at each site. The data were analyzed according to the National Committee for Clinical Laboratory Standards (NCCLS). The mean optical density (OD) and mean index value, standard deviation (SD), percent coefficient of variation (%CV), within run precision (intraassay) and within site (inter-assay) precision for each panel member at each site are illustrated below in the following tables.

Site 1

Panel Member	Neg		Low Pos		Pos #1		Pos #2		High Pos#1		High Pos #2		Neg Control		CO Control		Pos Control	
	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index
N	9	9	9	9	9	9	9	9	9	9	9	9	3	3	6	6	3	3
Mean	0.052	0.09	0.445	0.74	0.702	1.17	0.931	1.563	1.227	2.06	2.887	4.83	0.046	0.08	0.606	1.00	2.216	3.67
Within Run (intra-assay)[1] SD	0.002	0.00	0.022	0.03	0.059	0.09	0.044	0.08	0.051	0.09	0.089	0.17	N/A	N/A	0.02	0.03	N/A	N/A
%CV	N/A	N/A	4.8%	4.4%	8.4%	7.6%	4.7%	5.1%	4.2%	4.4%	3.1%	3.6%	N/A	N/A	3.7%	3.4%	N/A	N/A
Total (inter-assay)[2] SD	0.036	0.04	0.051	0.08	0.070	0.14	0.044	0.25	0.058	0.29	0.169	0.58	N/A	N/A	0.102	0.03	0.317	0.12
%CV	N/A	N/A	11.5%	10.4%	10.0%	11.6%	4.7%	15.7%	4.7%	14.3%	5.9%	11.9%	N/A	N/A	16.9%	2.8%	14.3%	3.3%

Site 2

Panel Member	Neg		Low Pos		Pos #1		Pos #2		High Pos#1		High Pos #2		Neg Control		CO Control		Pos Control	
	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index
N	9	9	9	9	9	9	9	9	9	9	9	9	3	3	6	6	3	3
Mean	0.040	0.10	0.280	0.70	0.364	0.89	0.602	1.49	0.801	2.01	1.361	3.43	0.074	0.18	0.415	1.00	1.197	2.97
Within Run (intra-assay)[1] SD	0.006	0.01	0.041	0.09	0.023	0.07	0.045	0.11	0.046	0.10	0.047	0.11	N/A	N/A	0.00	0.01	N/A	N/A
%CV	N/A	N/A	14.5%	13.0%	6.4%	7.6%	7.5%	7.1%	5.7%	4.8%	3.5%	3.2%	N/A	N/A	1.1%	1.1%	N/A	N/A
Total (inter-assay)[2] SD	0.006	0.03	0.058	0.19	0.083	0.18	0.057	0.28	0.042	0.53	0.079	1.00	N/A	N/A	0.094	0.01	0.068	0.54
%CV	N/A	N/A	20.8%	27.0%	22.7%	19.8%	9.5%	18.7%	5.3%	26.5%	5.8%	29.2%	N/A	N/A	22.7%	0.9%	5.7%	18.2%

Site 3

Panel Member	Neg		Low Pos		Pos #1		Pos #2		High Pos#1		High Pos #2		Neg Control		CO Control		Pos Control	
	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index
N	6	6	6	6	6	6	6	6	6	6	6	6	3	3	6	6	3	3
Mean	0.049	0.10	0.388	0.81	0.652	1.36	0.830	1.73	1.158	2.41	2.378	4.96	0.059	0.12	0.480	1.00	1.652	3.45
Within Run (intra-assay)[1] SD	0.003	0.01	0.009	0.02	0.082	0.17	0.068	0.14	0.094	0.20	0.126	0.25	N/A	N/A	0.028	0.06	N/A	N/A
%CV	N/A	N/A	2.4%	2.4%	12.5%	12.2%	8.2%	8.2%	8.1%	8.2%	5.3%	5.1%	N/A	N/A	5.8%	5.8%	N/A	N/A
Total (inter-assay)[2] SD	0.012	0.03	0.078	0.13	0.068	0.15	0.104	0.25	0.082	0.15	0.111	0.34	N/A	N/A	0.028	0.04	0.056	0.23
%CV	N/A	N/A	20.0%	15.8%	10.5%	11.1%	12.5%	14.3%	7.1%	6.2%	4.7%	6.8%	N/A	N/A	5.8%	4.1%	3.4%	6.6%

N/A = not applicable

[1]NCCLS EP5-A, Vol. 19, No. 2, Page 24, Equation (C2)

[2]NCCLS EP5-A, Vol. 19, No. 2, Page 25, Equation (C3) and Equation (C4)

B. Cross Reactivity

A study to evaluate the effect of potentially interfering medical conditions unrelated to Invasive Aspergillosis was performed with one lot of the Platelia™ *Aspergillus* EIA kit. The following serum samples were tested for cross-reactivity with the Platelia™ *Aspergillus* EIA. A total of 151 sera were tested.

Pathology	# Samples Tested	# Positives
Rheumatoid Factor	10	0
ANA Positive	10	0
IgG Hypergammaglobulinemia	10	0
IgM Hypergammaglobulinemia	10	0
Cancer*	11	0
Non-Viral Cirrhosis (primary biliary; alcohol induced; drug induced)	10	0
Multiple Transfusions	10	0
Multiparous Females	10	0
HAV	10	0
HCV	10	0
Rubella	10	0
CMV	10	0
Syphilis (RPR+)	10	0
Toxoplasmosis	10	0
Mycoplasma	10	0

* One each of bladder, breast(2), colon, endometrial, lung, prostate,renal, and squamous(3).

C. Clinical Testing

Clinical testing to evaluate the sensitivity, specificity, and predictive value of the Platelia™ *Aspergillus* EIA was conducted at two sites located in Belgium and the Netherlands. The study was conducted retrospectively using a total of 4873 serum samples collected from 203 patients from the following populations*:

- patients without signs of Invasive Aspergillosis (control patients)
- patients with Probable Invasive Aspergillosis
- patients with Proven Invasive Aspergillosis

* The Invasive Fungal Infection Cooperative Group (IFICG) of the European Organization for Research and Treatment of Cancer (EORTC) and the Mycosis Study Group (MSG) of the National Institute of Allergy and Infectious Diseases (NIAID) have defined criteria for diagnosis of Invasive Aspergillosis (IA) in patients with hematologic malignancy or hematopoetic stem cell transplant [2].

Proven Invasive Aspergillosis *is defined by positive microbiological culture obtained by sterile procedure from the site affected, and histopathological demonstration of the appropriate morphological forms in a host with symptoms attributed to the fungal infection.*

Probable Invasive Aspergillosis *is defined as at least one microbiological criterion,* ***and*** *one major or two minor clinical criteria from a site consistent with infection, in a host with symptoms attributed to the fungal infection.*

Possible Invasive Aspergillosis *is defined as at least one microbiological criterion,* ***or*** *one major or two minor clinical criteria from a site consistent with infection, in a host with symptoms attributed to the fungal infection.*

Given the relative rarity of Probable and Proven Invasive Aspergillosis, we offer the following definition of clinical sensitivity and specificity for the purposes of this study.

SENSITIVITY

Results from this study have been analyzed in terms of patient sensitivity. Sensitivity testing was conducted using the Platelia™ *Aspergillus* EIA at two sites on a combined total of 38 episodes from 35 Bone Marrow Transplant (BMT) and Leukemia patients diagnosed with Proven or Probable Invasive Aspergillosis.

1. Proven Aspergillosis (as defined by IFICG / EORTC ; see above)

Combined Sites **N = 19 episodes from 16 patients**
Sensitivity: 100% (19/19).
Note: The 95% confidence interval could not be calculated due to insufficient sample size.

2. Probable Aspergillosis (as defined by IFICG / EORTC ; see above)

Combined Sites **N = 19 episodes from 19 patients**
Sensitivity: 94.7% (18/19).
Note: The 95% confidence interval could not be calculated due to insufficient sample size.

3. Combined Proven and Probable Aspergillosis (as defined by IFICG / EORTC ; see above)

Combined Sites **N = 38 episodes from 35 patients**
Sensitivity: 97.4% (37/38). The 95% confidence interval is 86.2 – 99.9%.

SPECIFICITY

Specificity testing was conducted using the Platelia™ *Aspergillus* EIA at two sites on a combined total of 3691 samples obtained from 201 episodes (168 patients) without signs of Invasive Aspergillosis (control patients).

Site 1 : N = 3060 samples from 103 episodes (70 patients)
Specificity : 92.2% (95/103) The 95% confidence interval is 85.3 – 96.6%.

Site 2 N = 631 samples from 98 episodes (98 patients)
Specificity : 88.8% (87/98) The 95% confidence interval is 80.8 - 94.3%.

Combined Sites 3691 samples from 201 episodes (168 patients)
Specificity : 90.5% (182/201) The 95% confidence interval is 85.6 – 94.2%.

PREDICTIVE VALUE

Positive and Negative Predictive Values (PPV, NPV) have been analyzed for the patient population in this study, based on the actual average 16% prevalence rate observed in this study.
PPV: 66.1% (37/56)
NPV: 99.4% (182/183)
Analysis was also performed considering positive results when 2 consecutive samples had an index superior or equal to 0.5.
Performance is summarized in the following table:

	Sensitivity*	Specificity	PPV	NPV
Combined sites considering 1 sample ≥ 0.5	97.4% (37/38)	90.5% (182/201)	66.1% (37/56)	99.4% (182/183)
Combined sites considering 2 consecutive samples ≥ 0.5	92.1% (35/38)	97.5% (196/201)	87.5% (35/40)	98.5% (196/199)

*: sensitivity was calculated on Proven and Probable Invasive Aspergillosis

16- 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.

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CE

(US) - CE marking (European directive 98/79/CE on *in vitro* diagnostic medical devices)
(F) - Marquage CE (Directive européenne 98/79/CE relative aux dispositifs médicaux de diagnostic *in vitro*)
(E) - Marcado CE (Directiva europea 98/79/CE sobre productos sanitarios para diagnóstico *in vitro*)
(I) - Marchiatura CE (Direttiva europea 98/79/CE relativa ai dispositivi medico-diagnostici *in vitro*)
(D) - CE Konformitätskennzeichnung (Europäische Richtlinie 98/79/EG über *In-vitro*-Diagnostika)
(P) - Marcação CE (Directiva europeia 98/79/CE relativa aos dispositivos médicos de diagnóstico *in vitro*)
(S) - CE-märkning (Europeiskt direktiv 98/79/EG om medicintekniska produkter för *in vitro*-diagnostik)
(DK) - CE-mærkningen (Europa direktiv 98/79/EF om medicinsk udstyr til *in vitro*-diagnostik)
(GR) - Χαρακτηρισμος CE (ευρωπαικη οδηγια 98/79/CE περι *in vitro* διαγνωστικες ιατρικες συσκευες)
(PL) - CE oznaczenie (Dyrektywa unijna 98/79/CE dotycząca produktów medycznych do badań *in vitro*)
(LT) - CE ženklas (Europos sąjungos direktyva 98/79/CE dėl *in vitro* diagnostikos medicinos prietaisų)
(H) - CE jelzés (98/79/CE Európai Irányelv az *in vitro* orvosi diagnosztikai eszközökről)
(EST) - CE märgistus (Euroopa direktiiv 98/79/CE *in vitro* diagnostikameditsiiniseadmete kohta)
(SK) - CE označenie o zhode (Európska direktíva 98/79/CE pre *in vitro* diagnostické zdravotnícke postupy)
(CZ) - CE značka (Evropská direktiva 98/79/CE o diagnostických zdravotnických prostředcích *in vitro*)
(N) - CE-merking (EU-direktiv 98/79/CE om medisinsk utstyr til in vitro-diagnostikk)
(RO) - Marca CE (Directiva europeana 98/79/CE pentru dispozitive medicale de diagnostic *in vitro*)
(BG) - CE маркировка (Европейска директива 98/79/CE за ин витро диагностичните медицински изделия)

IVD

(US) - For *in vitro* diagnostic use
(F) - Pour diagnostic *in vitro*
(E) - Para diagnóstico *in vitro*
(I) - Per uso diagnostico *in vitro*
(D) - In-vitro-Diagnostikum
(P) - Para uso em diagnóstico *in vitro*
(S) - *In vitro*-diagnostik
(DK) - *In vitro* diagnose
(GR) - Για *in vitro* διαγνωστικη χρηση
(PL) - Do stosowania *in vitro*
(LT) - *in vitro* diagnostikai
(H) - Csak *in vitro* diagnosztikai alkalmazásra
(EST) - *In vitro* diagnostiliseks kasutamiseks
(SK) - Na diagnostiku *in vitro*
(CZ) - Pro diagnostiku *in vitro*
(N) - Til in vitro-diagnostikk
(RO) - Pentru diagnostic *in vitro*
(BG) - За ин витро диагностика

REF

(US) - Catalogue number
(F) - Référence catalogue
(E) - Número de catálogo
(I) - Numero di catalogo
(D) - Bestellnummer
(P) - Número de catálogo
(S) - Katalognummer
(DK) - Katalognummer
(GR) - Αριθμος καταλογου
(PL) - Numer katalogu
(LT) - Katalogo numeris
(H) - Cikkszám
(EST) - Katalooginumber
(SK) - Katalógové číslo
(CZ) - Katalogové číslo
(N) - Katalognummer
(RO) - Număr de catalog
(BG) - Каталожен номер

(US) - Manufacturer
(F) - Fabricant
(E) - Fabricante
(I) - Produttore
(D) - Hersteller
(P) - Fabricante
(S) - Tillverkad av
(DK) - Fremstillet af
(GR) - Κατασκευαστης
(PL) - Producent
(LT) - Gamintojas
(H) - Gyártó
(EST) - Tootja
(SK) - Výrobca
(CZ) - Výrobce
(N) - Produsent
(RO) - Producător
(BG) - Производител

EC REP

(US) - Authorised Representative
(F) - Représentant agréé
(E) - Representante autorizado
(I) - Distributore autorizzato
(D) - Bevollmächtigter
(P) - Representante Autorizado
(S) - Auktoriserad representant
(DK) - Autoriseret repræsentant
(GR) - Εξουσιοδοτημενος αντιπροσωπος
(PL) - Upoważniony Przedstawiciel
(LT) - Įgaliotasis atstovas
(H) - Meghatalmazott Képviselő
(EST) - Volitatud esindaja
(SK) - Autorizovaný zástupca
(CZ) - Zplnomocněný zástupce
(N) - Autorisert representant
(RO) - Reprezentant autorizat
(BG) - Упълномощен представител

LOT

(US) - Batch code
(F) - Code du lot
(E) - Código de lote
(I) - Codice del lotto
(D) - Chargen-Bezeichnung
(P) - Código do lote
(S) - Batchnr
(DK) - Batchkoden
(GR) - Κωδικας παρτιδας
(PL) - Numer serii
(LT) - Serijos numeris
(H) - Gyártási szám
(EST) - Partii kood
(SK) - Číslo šarže
(CZ) - Číslo šarže
(N) - Partikode
(RO) - Număr de lot
(BG) - Партиден номер

(US) - Expiry date YYYY/MM/DD
(F) - Date de peremption AAAA/MM/JJ
(E) - Estable hasta AAAA/MM/DD
(I) - Da utilizzare prima del AAAA/MM/GG
(D) - Verwendbar bis JJJJ/MM/TT
(P) - Data de expiração AAAA/MM/DD
(S) - Utgångsdatum ÅÅÅÅ/MM/DD
(DK) - Anvendes før ÅÅÅÅ/MM/DD
(GR) - Ημερομηνια ληξης YYYY/MM/DD
(PL) - Data ważności YYYY/MM/DD
(LT) - Galioja iki YYYY/MM/DD
(H) - Szavatossági idő ÉÉÉÉ/HH/NN
(EST) - Aegumistähtaeg AAAA/KK/PP
(SK) - Použiteľné do RRRR/MM/DD
(CZ) - Datum exspirace RRRR/MM/DD
(N) - Utløpsdato ÅÅÅÅ/MM/DD
(RO) - Data expirarii AAAA/LL/ZZ
(BG) - Срок на годност година/месец/ден

(US) - Storage temperature limitation
(F) - Limites de températures de stockage
(E) - Temperatura límite
(I) - Limiti di temperatura di conservazione
(D) - Lagertemperatur
(P) - Limites de temperatura de armazenamento
(S) - Temperaturbegränsning
(DK) - Temperaturbegrænsning
(GR) - Περιορισμος θερμοκρασιας αποθηκευσης
(PL) - Temperatura przechowywania
(LT) - Saugojimo temperatūriniai apribojimai
(H) - Tárolási hőmérsékleti határok
(EST) - Piirangud säilitustemperatuurile
(SK) - Skladovacia teplota od do
(CZ) - Teplotní rozmezí od do
(N) - Oppbevaringstemperatur
(RO) - Limitele de temperatură la stocare
(BG) - Температурни граници на съхранение

(US) - Consult Instruction for use
(F) - Consulter le mode d'emploi
(E) - Consulte las instrucciones de uso
(I) - Consultare le istruzioni per uso
(D) - Siehe Gebrauchsanweisung
(P) - Consulte o folheto informativo
(S) - Se bruksanvisningen
(DK) - Se instruktion før brug
(GR) - Συμβουλευθειτε τις οδηγιες χρησης
(PL) - Sprawdź instrukcję
(LT) - Ieškokite informacijos vartojimo instrukcijoje
(H) - Olvassa el a használati utasítást
(EST) - Kasutamisel vaata instruktsiooni
(SK) - Katalógové číslo
(CZ) - Viz návod k použití
(N) - Se bruksanvisninger
(RO) - Consultati prospectul de utilizare
(BG) - Виж инструкцията за употреба

(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 Directiva 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 krävs 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) - Τις υπολοιπες γλωσσες που απαιτουνται για συμμορφωση στην ευρωπαικη οδηγια μπορειτε να τις προμηθευθειτε απο τον τοπικο σας αντιπροσωπο Bio-Rad.

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

(LT) - Vertimus, reikalingus pagal Europos sąjungos direktyvos reikalavimus, į kitas kalbas galite gauti iš vietinio Bio-Rad atstovo.

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

(EST) - Teised vastavalt Euroopa Direktiivile nõutavad keeled on saadaval kohaliku Bio-Radi edasimüüja käest.

(SK) - Ostatné jazykové 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.

(N) - Øvrige språk som kreves i henhold til EU-direktivet, fås fra din lokale Bio-Rad-representant.

(RO) - Alte traduceri cerute în conformitate cu Directiva Europeană se pot obține de la Reprezentanța Bio-Rad locală.

(BG) - Останалите езици, които се изискват съгласно Европейската Директива, могат да Ви бъдат предоставени от локалния представител на Био-Рад.

Bio-Rad
3, boulevard Raymond Poincaré
92430 Marnes-la-Coquette France
Tel. : +33 (0) 1 47 95 60 00
Fax.: +33 (0) 1 47 41 91 33

CE

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