clarus *Blastomyces* Total Ab EIA Test Kit

For the Detection of *Blastomyces* Antibodies – REF BTA101

IMMY
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IVD

Qualitative Screening Procedure

A. Dilute samples 1:441 in 1X Specimen Diluent.
B. 100 µL sample for 30 minutes (22-25° C); decant.
C. Wash 3 times.
D. 100 µL Conjugate for 30 minutes (22-25° C); decant.
E. Wash 3 times.
F. 100 µL Substrate for 10 minutes (22-25° C).
G. 100 µL Stop Solution.
H. Measure OD.

Interpretation of the Results

EIA Units	Interpretation
x < 1.0	Negative
1.0 ≤ x < 1.5	Indeterminate
1.5 ≤ x	Positive

INTENDED USE

The clarus *Blastomyces* Total Antibody Enzyme Immunoassay (EIA) is used for the qualitative detection of serum antibodies directed against yeast-phase antigens from *Blastomyces.*

The clarus *Blastomyces* Total Antibody 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 blastomycosis. (3)

EXPLANATION

Blastomyces dermatitidis is a thermally dimorphic fungus that causes the pulmonary pyogranulomatous disease, blastomycosis (1,3). Initial infection is through the lungs and although often subclinical, lymphohematogeneous dissemination may occur, culminating in systemic disease (1,3). Clinical disease most often involves the lungs, skin, bones, genitourinary system and central nervous system but dissemination can involve any organ. (3) *Blastomyces* is endemic in the southern and southeastern states that border the Ohio and Mississippi River valleys as well as Midwestern states and Canadian provinces that border the Great Lakes and the Saint Lawrence Riverway. (3) Additionally, autochthonous cases of blastomycosis have been diagnosed in Africa and India. (3) Serologic testing for blastomycosis should be requested when a patient shows signs of a respiratory infection that progresses gradually, with fever, weight loss, cough with sputum, chest pain, and night sweats. (1,3) The test should also be performed when there are verrucous or ulcerative lesions on the skin, a common sign of dissemination. (1,3)

Blastomycosis presents a diagnostic challenge as the manifestations of most early infections overlap substantially with those of other respiratory infections or malignancies (3). In addition, diagnosis by histological or culture studies, though ideal, may take time or reflect false results. (1,3) Therefore, specific laboratory testing is usually required to establish a diagnosis of blastomycosis. Serologic tests such as complement fixation (CF) and immunodiffusion (ID) have served for several decades as aids in the diagnosis and management of blastomycosis. (1,3) The CF assay lacks both sensitivity and specificity, and its performance is complex and labor-intensive. The CF assay exhibits low specificity due to cross-reactive antibodies recognizing carbohydrate moieties common to several fungi. Although the ID assay is more sensitive and specific than the CF assay, it takes 48 hours to perform and requires highly skilled personnel to properly interpret results. (1,3) The clarus *Blastomyces* Total Antibody EIA, however, is a sensitive, specific, and rapid test for the qualitative detection of serum antibodies against antigens from *B. dermatitidis*.

BIOLOGICAL PRINCIPLES

The clarus *Blastomyces* Total Antibody EIA utilizes a proprietary mixture of purified *Blastomyces* yeast-phase antigens adsorbed to microwells for the capture of serum antibodies. Patient specimens, diluted in the provided specimen diluent buffer, are added and incubated in antigen-coated microwells. If antibodies are present in patient specimens, they will become bound to the adsorbed antigens. The wells are washed to remove unbound patient material, and secondary antibody consisting of anti-human antibody conjugated to a horseradish peroxidase (HRP) enzyme is added. If patient antibodies are bound to the adsorbed antigens, the secondary antibody will become bound to the patient antibodies. Wells are again washed to remove any unbound secondary antibody and TMB substrate solution is then added. A blue color develops in the presence of the HRP enzyme. The reaction is stopped by the addition of a stop solution. Optical density (absorbance) is determined with a microplate reader at 450 nm alone or at 450 nm and 630 nm. Sample OD readings are compared to calibrator cutoff OD readings to determine results.

REAGENTS

A. **Antigen-Coated Microwells** (96) – REF BTAMW1 – Stripwell plates featuring breakaway polystyrene microwells coated with *Blastomyces* antigen.
B. **Positive Control** (1.5 mL x 1) – REF BTAPC1 - An anti-*Blastomyces* antibody in a buffered protein solution.
C. **Calibrator Cutoff** (1.5 mL x 1) – REF BTACC1 - An anti-*Blastomyces* antibody in a buffered solution that establishes the cutoff signal for the calculation of EIA units.
D. **10X Specimen Diluent** (10 mL x 1) – REF EIASD1 - Concentrated buffered protein solution containing a preservative.
E. **20X Wash Buffer** (50 mL x 1) – REF EIAWB1 - Concentrated wash buffer with a preservative.
F. **Enzyme Conjugate** (10 mL x 1) – REF BTAEZC - Affinity-purified rabbit anti-human antibodies conjugated to horseradish peroxidase (HRP) in a buffered blocking solution containing a mercury-free preservative.
G. **TMB Substrate** (10 mL x 1) – REF EIATMB - Buffered solution containing urea peroxide and tetramethylbenzidine (TMB).
H. **Stop Solution** (10 mL x 1) – REF EIASS1 - 2N sulfuric acid. CAUTION: AVOID CONTACT WITH SKIN. FLUSH WITH WATER IF CONTACT OCCURS.

REAGENT PRECAUTIONS

A. All reagents are intended for in vitro diagnostic use only!
B. Specific standardization is necessary to produce our high-quality reagents and materials. IMMY cannot guarantee the performance of its products when used with materials purchased from other manufacturers. Do not interchange reagents from different kit lot numbers or other manufacturers.
C. The user assumes full responsibility for any modification to the procedures published herein.
D. Always wear gloves when handling reagents in this kit as some reagents are preserved with 0.095% (w/w) sodium azide. Sodium azide should not be flushed down the drain, as this chemical may react with lead or copper plumbing to form potentially explosive metal azides. Excess reagents should be discarded in an appropriate waste receptacle.
E. Avoid contact with Stop Solution (2 N sulfuric acid). If exposed, immediately flush with copious amounts of water.
F. Avoid splashing when dispensing reagents into the microwells as this causes erroneous results.
G. Inadequate washing can cause excessive background reactivity in any EIA protocol.
H. Use only protocols described in this package insert. Incubation times or temperatures other than those specified may give erroneous results.
I. Maintain proper pipetting techniques and pattern throughout procedure to ensure optimal and reproducible results.

REAGENT PREPARATIONS

A. The entire kit, including the microwell plate, should be at room temperature (22-25° C) before and during use.
B. Prepare a 1X solution of Specimen Diluent by mixing 9 parts DI water with 1 part 10X Specimen Diluent.
C. Prepare a 1X solution of Wash Buffer by mixing 19 parts DI water with 1 part 20X Wash Buffer.

REAGENT STABILITY AND STORAGE

The entire clarus *Blastomyces* Total Antibody EIA test kit should be stored at 2-8° C until the expiration dates listed on the reagent labels. All reagents should be returned to the refrigerator promptly after use.

Unused microwells should be placed in the resealable Mylar bag and sealed immediately after opening. Care should be taken to ensure the desiccant pouch remains in the bag with unused microwells. Store at 2-8° C.

SPECIMEN COLLECTION AND PREPARATION

Collect samples aseptically using established techniques by qualified personnel. When handling patient specimens, adequate measures should be taken to prevent exposure to potentially present etiologic agents. The use of specimens other than serum has not been established. Hyperlipemic, hemolyzed, icteric, and/or contaminated samples must not be tested.

For optimal results, sterile samples should be used. If a delay is encountered in specimen processing, storage at 2-8° C for up to 72 hours is permissible. Specimens may be stored for longer periods at <-20° C, provided they are not repeatedly thawed and refrozen, as this may affect test results (do not store in a frost-free freezer). Specimens in transit between labs should be maintained at 2-8° C or <-20° C. (2)

Specimens should be brought to room temperature (22 to 25° C) prior to testing.

Dilute the serum specimens with the 1X Specimen Diluent as described below. Inaccurate dilutions can be a source of error in any EIA procedure.

Dilute serum 1:441 with 1X Specimen Diluent as follows:

A. Obtain two test tubes for each serum specimen. Transfer 200 µL of 1X Specimen Diluent to the first tube and 400 µL to the second tube.
B. Transfer 10 µL of serum to the first tube and mix thoroughly.
C. Transfer 20 µL of the first dilution into the second tube and mix thoroughly.

PROCEDURE

REFER TO *REAGENTS* SECTION FOR A LIST OF MATERIALS PROVIDED.

MATERIALS NOT PROVIDED

A. Pipets capable of delivering up to 200 µL and disposable tips
B. Distilled or deionized water
C. Spectrophotometer microplate reader capable of reading absorbances at 450 nm or 450 and 630 nm with software capable of generating a four-parameter curve
D. Squirt bottle, EIA plate washer, or multichannel pipettor for washing
E. Timer
F. Graduated cylinder for dilutions of wash buffer and specimen diluent
G. Test tubes for specimen dilution

QUALITATIVE SCREENING PROCEDURE

A. **Bring all kit components to room temperature.**

B. Snap off a sufficient number of microwells (BTAMW1) for samples and controls and insert them into the microwell holder. Record the position of each patient and the controls.
C. Add 100 µL of Positive Control (BTAPC1) to a microwell.
D. Add 100 µL of 1X Specimen Diluent (Negative Control) to a microwell.
E. Add 100 µL of Calibrator Cutoff (BTACC1) to a microwell. This well will indicate the cutoff absorbance for the calculation of EIA units.
F. Add 100 µL of diluted specimen to a microwell.
G. Mix by gently shaking 1-5 seconds on the countertop.
H. Incubate plate at room temperature (22-25° C) for 30 minutes.
I. Using a pipettor, aspirate the contents from the wells and discard into a biohazard receptacle.
J. Dispense at least 200uL of 1X Wash Buffer into all wells.
K. Repeat step J two more times for a total of three washes. After the final wash, strike the plate on a clean stack of paper towels or other absorbent material firmly enough to remove as much wash buffer as possible.
L. Add 100 µL of the Enzyme Conjugate (BTAEZC) to each of the wells.
M. Mix by gently shaking 10-15 seconds on the countertop.
N. Incubate plate at room temperature (22-25° C) for 30 minutes.
O. Repeat steps I-K.
P. Add 100 µL of Substrate (EIATMB) to each microwell. Start a timer with the addition of the Substrate to the first well.
Q. Mix by gently shaking 10-15 seconds on the countertop.
R. Incubate the plate at room temperature (22-25° C) for 10 minutes.
S. Add 100 µL of Stop Solution (EIASS1) to each microwell in the same order as step P.
T. Mix by gently shaking 10-15 seconds on the countertop.
U. Read the test and record results.

READING THE TEST

A. The plate can be read at this time. The reading should take place within 15 minutes of test completion. Carefully wipe the undersides of the wells with a clean, lint-free tissue and measure the absorbance of each microwell as outlined below.
   1. A dual wavelength reader is preferred, with absorbances read 450 nm and 630 nm. Blank on negative control.
   2. If a single wavelength reader is used, read the absorbance at 450 nm. Blank on negative control.
B. Calculate EIA units by dividing the absorbance value for each well by the absorbance value of the Calibrator Cutoff well.
C. Disinfect and retain microwell holder. Discard used assay materials as biohazardous waste.

QUALITY CONTROL

It is recommended that until the user becomes familiar with the kit performance, all specimens and controls be run in duplicate. The Positive Control, Negative Control, and Calibrator Cutoff must be assayed with each batch of patient specimens to provide quality assurance of the reagents. The Positive and Negative controls are intended to monitor for substantial reagent failure. The Positive Control should not be used as an indicator of Calibrator Cutoff precision and only ensures reagent functionality. The Calibrator Cutoff has been formulated to give the optimum differentiation between negative and positive sera. Although the absorbance value may vary between runs and laboratories, the mean value for the Calibrator Cutoff must be within 0.175 to 0.350 OD units.

Report results as index values (i.e. EIA units) relative to the Calibrator Cutoff. The Positive Control EIA units should be between 2 and 6. The Negative Control EIA units should be less than 1.

If the Calibrator Cutoff, Positive Control, and/or Negative Control are not within these parameters, patient test results should be considered invalid and the assay repeated.

At the time of each use, the kit components should be visually inspected for obvious signs of microbial contamination, freezing, or leakage.

RESULTS

Calculate EIA units by dividing the absorbance value of each specimen well by the absorbance value of the Calibrator Cutoff well.

$$EIA\ Units = \frac{Blanked\ OD\ of\ Specimen}{Blanked\ OD\ of\ Calibrator\ Cutoff}$$

SAMPLE CALCULATION

$$EIA\ Units = \frac{0.350}{0.175} = 2.0\ EIA\ Units$$

INTERPRETATION OF RESULTS

EIA Units	Interpretation
x < 1.0	Negative
1.0 ≤ x < 1.5	Indeterminate
1.5 ≤ x	Positive

Specimen testing should be repeated if the results are indeterminate or low-positive and inconsistent with clinical findings.

LIMITATIONS OF THE PROCEDURE

The clarus *Blastomyces* Total Antibody EIA is intended for use with serum specimens only. The performance characteristics of this assay have not been evaluated for other types of specimens.

A negative result does not preclude a diagnosis of blastomycosis, particularly if only a single specimen has been tested and the patient shows symptoms consistent with a positive diagnosis.

A positive result implies the presence of serum antibodies to *Blastomyces.*

EXPECTED VALUES AND PERFORMANCE CHARACTERISTICS

EXPECTED VALUES

Positive Predictive Value (PPV) and Negative Predictive Value (NPV)		Disease*			
		Positive	Negative		
Test	Pos.	13	0	Total Pos. 13	PPV 100% (13/13)
	Neg.	0	136	Total Neg. 136	NPV 100% (136/136)
		Total Pos. 13	Total Neg. 136		
		Sensitivity 100%	Specificity 100%		

Indeterminate results were excluded from the sensitivity, specificity, PPV, and NPV calculations. Calculations were based on a comparison with a normal population. *Patients were classified as positive for *Blastomyces* when an immunodiffusion (ID) test yielded an identity band or *Blastomyces* was confirmed by culture identification.

RELATIVE SENSITIVITY AND SPECIFICITY (ID- OR CULTURE-POSITIVE), n= 268

The relative sensitivity and specificity of the clarus *Blastomyces* Total Antibody EIA were evaluated versus *Blastomyces* immunodiffusion (ID) testing or culture confirmation. A total of 268 serum specimens were tested using the clarus *Blastomyces* Total Antibody EIA. The sample set consisted of 137 samples representing a normal population, 15 *Blastomyces* ID or culture-positive samples, and 116 samples from patients identified as positive for other fungal infections by immunodiffusion (ID) or complement fixation (CF) testing.

		IMMY clarus *Blasto* Total Ab EIA Results		
Characteristic	n	Neg.	Ind.	Pos.
Relative sensitivity with *Blastomyces* ID- or culture-positive patients	15	0% (0/15)	13.3% (2/15)	86.7% (13/15)
Relative specificity with normal population	137	99.3% (136/137)	0.7% (1/137)	0% (0/137)
Cross-reactivity with patients positive for other fungal infections (ID- or CF-positive for *Aspergillus, Coccidioides,* or *Histoplasma)*	116	97.4% (113/116)	1.7% (2/116)	0.9% (1/116)

Due to the equivocal nature of low titer CF-positive results, the data was analyzed to determine sensitivity and specificity for data subsets consisting of *Blastomyces* CF-positive specimens categorized according to titer. When the CF titers of the *Blastomyces*-positive specimens were greater than or equal to 1:16, which was the case in 5 of the 6 *Blastomyces* specimens, 100% of the specimens were identified as positive.

When the CF titers of the *Blastomyces*-positive specimens were greater than or equal to 1:8, 83.3% of the samples were identified as positive. One of the specimens with a CF titer of 1:8 or greater was identified as negative.

		IMMY clarus *Blasto* Total Ab EIA Results		
Blasto CF Titer	n	Negative	Indeterminate	Positive
≥ 1:8	6	16.7% (1/6)	0% (0/6)	83.3% (5/6)
≥ 1:16	5	0% (0/5)	0% (0/5)	100% (5/5)

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3. Saccente, M. and G. L. Woods. 2010. Clinical and laboratory update on blastomycosis. Clin.Microbiol.Rev. **23**:367-381.

INTERNATIONAL SYMBOL USAGE

8°C 2°C	Storage 2-8 C	LOT	Lot Number
	Manufactured by	REF	Reference Number
	Expiration Date	IVD	In Vitro Diagnostics
	Protect from Humidity	Σ	Sufficient for "#" Tests

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