clarus

COCCIDIOIDES Ab

ENZYME IMMUNOASSAY

For the Qualitative Detection of *Coccidioides* Antibody – REF CAB102

℞ ONLY

IVD For In Vitro Use Only

2°C – 8°C

IMMY

TABLE OF CONTENTS

Rev. 3
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INTENDED USE

The clarus *Coccidioides* Antibody Enzyme Immunoassay (EIA) is used for the qualitative detection of IgM and IgG antibodies directed against TP and CF antigens from *Coccidioides* species as an aid in the diagnosis of coccidioidomycosis in serum and cerebrospinal fluid (CSF).

SUMMARY AND EXPLANATION OF THE TEST

Coccidioidomycosis is an airborne, infectious disease that is caused by the *Coccidioides* spp. organisms.[1] *Coccidioides* are dimorphic fungi that thrive in arid desert soils and environments with mild winters and dry summers.[1] Exposure occurs when these microscopic spores are inhaled into the lungs.[2] Infection can lead to respiratory diseases, and occasionally, diseases affecting other systems.[3] Though endemic in the southwestern United States and Mexico, increased travel to the endemic areas has also increased the incidence in non-endemic areas.[2] Coccidioidomycosis should be considered whenever patients display flu-like symptoms and have lived or traveled to the endemic areas.[3]

Coccidioidomycosis presents a diagnostic challenge to the physician and laboratorian. The symptoms of most early coccidioidal infections substantially mimic those of other respiratory infections.[4] In addition, the organisms can be difficult to demonstrate via culture and histologically, even after repeated attempts.[5] Therefore, specific laboratory testing is usually required to establish a diagnosis of coccidioidomycosis.

Serologic tests have served for several decades as aids in the diagnosis and management of coccidioidomycosis.[6] Complement fixation (CF), immunodiffusion (ID), and latex agglutination (LA) have been the most commonly used serologic methods. The CF assay is sensitive; however, its performance is complex and labor-intensive. Additionally, the CF assay exhibits low specificity due to cross-reactive antibodies that recognize carbohydrate moieties common to several fungi. The ID assay is more specific but less sensitive than the CF assay. Additionally, the ID assay takes 48 hours to perform and requires highly skilled personnel to properly interpret results. The LA assay is sensitive and rapid but lacks specificity. However, the clarus *Coccidioides* Antibody EIA (CAB102) is a sensitive, specific, and rapid test for the qualitative detection of IgM (TP antigen) and IgG (CF antigen) antibodies from *Coccidioides*.

BIOLOGICAL PRINCIPLES

The CAB102 is an immunoenzymatic, sandwich microplate assay that detects IgM and IgG antibodies in serum and cerebrospinal fluid (CSF). It uses a proprietary mixture of recombinant and native *Coccidioides* antigens, including the CF and TP antigens, adsorbed to microwells. The high sensitivity and specificity of this test are achieved through the utilization of different *Coccidioides* antigen preparations for the detection of antibodies. IgM Antibodies (typically against TP antigens) are formed early in the course of the disease and are followed by IgG antibodies (typically against CF antigen) as the disease progresses.[7] Diluted patient specimens and controls are incubated in both TP and CF microwells. If antibodies against *Coccidioides* are present in patient specimens, the antibodies will become bound to the adsorbed antigens.

Nonspecific reactants are removed during the washing step. After the first wash, a peroxidase-conjugated secondary anti-human antibody is added to the microwells. If patient antibodies are bound to the adsorbed antigens, the peroxidase-conjugated secondary antibody will become bound to the patient antibodies. Excess peroxidase-conjugated secondary antibody is removed by a second washing step. After the second wash step, a substrate solution is then added to the microwells, developing color in the presence of peroxidase-conjugated secondary antibody. Adding Stop Solution halts the reactivity of the substrate and the color change is quantified by measuring optical density (OD). Sample OD readings are compared to calibrator cutoff OD readings to determine results.

REAGENTS

REAGENT	REF#	QTY	DESCRIPTION	Label Symbol	Hazard Symbol
10X Specimen Diluent	EIASD1	20 mL	Concentrated, buffered protein solution with a preservative.	1	
20X Wash Buffer	EIAWB1	50 mL	Concentrated wash buffer with a preservative.	2	N/A
CF Antigen-Coated Microwells	CABMWG	96	Color-coded (blue = CF) microwell plate featuring breakaway polystyrene microwells.	3	N/A
TP Antigen-Coated Microwells	CABMWM	96	Color-coded (clear = TP) microwell plate featuring breakaway polystyrene microwells.	4	N/A
CF Calibrator Cutoff	CABCCG	1.5 mL x2	Anti-*Coccidioides* CF antibodies in a buffered protein solution (with a preservative) for establishing the cutoff signal for calculating CF EIA units.	5	N/A
TP Calibrator Cutoff	CABCCM	1.5 mL x2	Anti-*Coccidioides* TP antibodies in a buffered protein solution (with a preservative) for establishing the cutoff signal for calculating TP EIA units.	6	N/A
CF Enzyme Conjugate	CABIGG	10 mL	Affinity-purified rabbit anti-human IgG antibodies conjugated to horseradish peroxidase (HRP) in a buffered protein solution with a preservative.	7	N/A
TP Enzyme Conjugate	CABIGM	10 mL	Affinity-purified rabbit anti-human IgM antibodies conjugated to horseradish peroxidase (HRP) in a buffered protein solution with a preservative.	8	N/A
TMB Substrate	EIATMB	20 mL	Buffered solution containing urea peroxide and tetramethylbenzidine. TMB substrate is light-sensitive and should be kept out of direct light	9	N/A
Stop Solution	EIASS1	20 mL	2 N sulfuric acid. CAUTION: AVOID CONTACT WITH SKIN. FLUSH WITH WATER IF CONTACT OCCURS.	10	
Positive Control	CABPC1	1.5 mL x2	Anti-*Coccidioides* antibodies in a buffered protein solution containing a preservative.	+	N/A
Microwell Strip Holder		2			

REAGENT STORAGE AND STABILITY

- The entire CAB102 test kit should be stored at 2-8 °C until the expiration dates listed on the labels. All reagents not used during testing should be returned to 2-8 °C storage promptly after use.
- Unused microwells (3 / 4) should be placed in the resealable Mylar bags and sealed immediately after opening and stored at 2-8 °C. Care should be taken to ensure the desiccant pouch remains in the bag with unused microwells.

REAGENT PREPARATIONS

- The entire kit, including the microwell plate, should be at 20-25 °C before and during use. Warming requires at least one hour.
- Prepare a 1X solution of Specimen Diluent by mixing 9 parts DI water with 1-part 10X Specimen Diluent (1). 1X Specimen Diluent is stable for one week when stored at 2-8°C.
- Prepare a 1X solution of Wash Buffer by mixing 19 parts DI water with 1-part 20X Wash Buffer (2). 1X Wash Buffer is stable for 1 month when stored at 2-8°C.

**Refer to SDS for complete hazard information.*

MATERIALS REQUIRED BUT NOT PROVIDED

A. Pipettor capable of delivering ranges up to 200 µL and disposable tips
B. Test tubes for dilution of specimens
C. Distilled or deionized water
D. Spectrophotometer plate reader (Dual ODs at A = 450 nm and 630 nm)
E. EIA plate washer or multi-channel pipettor for washing
F. Timer
G. Graduated cylinders for dilutions of wash buffer and specimen diluent

WARNINGS AND 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 or aspirating reagents into the microwells as this causes errors.
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 the procedure to ensure optimal and reproducible results.

SPECIMEN COLLECTION AND PREPARATION

Using established techniques by qualified personnel, collect samples aseptically. When handling patient specimens, adequate measures should be taken to prevent exposure to potential etiologic agents. This assay has not been validated on specimens other than serum and CSF.

For optimal results, sterile samples should be used. Specimens should be tested as soon as possible but may be stored for up to 5 days at 2-8 °C prior to testing. If longer storage is required, several aliquots of each specimen should be frozen (-20 to -80 °C) to avoid multiple freeze-thaw cycles. Do not store in a frost-free freezer.

Prior to specimen dilution, specimens should be brought to 20-25 °C.

Use patient samples as soon as possible after diluting with 1X Specimen Diluent (1).

Note: Please be sure to use the correct dilution for your specimen type.

SERUM

Dilute to 1:441 with 1X Specimen Diluent as follows:	
STEP 1	Obtain 2 test tubes for each serum specimen. Transfer 200 µL of 1X Specimen Diluent to the first tube and 400 µL to the second tube.
STEP 2	Transfer 10 µL of patient serum to the first tube and mix thoroughly.
STEP 3	Transfer 20 µL of the first dilution into the second tube and mix thoroughly.

CSF

Dilute to 1:21 with 1X Specimen Diluent as follows:	
STEP 1	Obtain 1 test tube for each CSF specimen. Transfer 200 µL of 1X Specimen Diluent to each tube.
STEP 2	Transfer 10 µL of patient CSF to the tube and mix thoroughly.

TEST PROCEDURE

QUALITATIVE SCREENING PROCEDURE

CF microwells – blue
TP microwells – clear

Step 1	Aliquot enough reagents necessary for tests being run that day, then return the remaining reagents to cold storage *(NOTE: When aliquoting TMB Substrate (9), protect the reagent from light)*
Step 2	Bring all kit components to 20-25 °C
Step 3	Snap off a sufficient number of Antigen-Coated Microwells (3/4) for patient samples, standards, and controls and insert them into the microwell holder, recording the position of each sample, standard, and control. *(NOTE: Place remaining microwells back into bag with desiccant and store at 2-8 °C.)*
Step 4	Dispense 100 µL of each diluted specimen into both blue (CF) and clear (TP) microwells.
Step 5	Dispense 100 µL of Positive Control (+) to a blue (CF) well and 100 µL to a clear (TP) well. These microwells will be the positive controls for the assay.
Step 6	Dispense 100 µL of 1X Specimen Diluent (1) to a blue (CF) well and 100 µL to a clear (TP) well. These microwells will be the negative controls for the assay.
Step 7	Dispense 100 µL of 1X Specimen Diluent (1) to a blue (CF) well and 100 µL to a clear (TP) well. These microwells will be the blanks for the assay.
Step 8	Dispense 100 µL of CF Calibrator Cutoff (5) to a blue (CF) well and 100 µL of TP Calibrator Cutoff (6) to a clear (TP) well. These microwells will indicate the cutoff ODs for the calculations of EIA units.
Step 9	If running manually, gently shake 1-2 seconds to ensure reagent is at bottom of well (optional).
Step 10	Incubate plate at room temperature (20-25 °C) for 30 minutes ± 5 minutes.

Step 11	Using a pipettor, aspirate the contents from the wells and discard into a biohazard receptacle.
Step 12	Fill microwells with 200 µL – 300 µL of 1X Wash Buffer (2). This can be accomplished using an EIA plate washer or multichannel pipettor. Dump the plate contents.
Step 13	Repeat step 12 2 more times for a total of 3 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.
Step 14	Dispense 100 µL of the CF Enzyme Conjugate (7) to each of the blue (CF) microwells.
Step 15	Dispense 100 µL of the TP Enzyme Conjugate (8) to each of the clear (TP) microwells.
Step 16	If running manually, gently shake 1-2 seconds (optional).
Step 17	Incubate plate at room temperature (20-25 °C) for 30 minutes ± 5 minutes.
Step 18	Repeat steps 11-13.
Step 19	Dispense 100 µL of TMB Substrate (9) to each microwell. Start a timer for 10 minutes with the addition of the substrate to the first well.
Step 20	If running manually, gently shake 1-2 seconds to ensure reagent is at bottom of well (optional).
Step 21	Incubate the plate at room temperature away from direct light (20-25 °C) for remainder of 10 minutes ± 1 minute.
Step 22	Dispense 100 µL of Stop Solution (10) to each microwell in the same order as step 19.
Step 23	If running manually, gently shake 1-2 seconds to ensure reagent is at bottom of well (optional).
Step 24	Read and record results (see READING THE TEST).

READING THE TEST

A. Reading the plate should take place within 15 minutes of test completion.
B. Carefully wipe the undersides of the microwells with a clean, lint-free tissue, and measure the OD of each microwell as outlined below.
    1. A dual-wavelength reader is preferred, with ODs read 450 nm and 630 nm. Blank on blank microwells (refer to Qualitative Screening Procedure, step 7).
C. Disinfect and retain microwell holder. Discard used assay materials as biohazard waste.
D. Proceed to Quality Control and Results section.

QUALITY CONTROL

At the time of each use, kit components should be visually inspected for obvious signs of microbial contamination, freezing, or leakage. Discard if these conditions are found.

It is recommended that until the user becomes familiar with the kit performance, all specimens and controls are run in duplicate. The positive control, negative control, and calibrator cutoffs 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. Calibrator cutoffs have been formulated to give the optimum differentiation between negative and positive sera. Although the OD values may vary between runs and laboratories, the mean value for the calibrator cutoffs and the positive control must be within:

CF Calibrator Cutoff	**Blanked OD between 0.100 – 0.250**
TP Calibrator Cutoff	**Blanked OD between 0.200 – 0.350**
CF Positive Control	EIA Units between 2.0 – 6.0
TP Positive Control	EIA Units between 2.0 – 6.0
Negative Control	EIA Units less than 1.0

If the EIA units for the calibrator cutoffs, positive control, or negative control are not within these parameters, patient test results should be considered invalid and the assay repeated.

RESULTS

Note: Please be sure to use the correct interpretation for your specimen type.

SERUM INTERPRETATION OF RESULTS

Calculate CF EIA units by dividing the blanked OD value of each blue (CF) well by the blanked OD value of the blue (CF) Calibrator Cutoff well. Calculate TP EIA units by dividing the blanked OD value of each clear (TP) well by the blanked OD value of the clear (TP) Calibrator Cutoff well.

CF EIA Units	TP EIA Units	Interpretation
< 1	< 1	Negative
≥ 1 to < 1.5	≥ 1 to < 1.5	Indeterminate
≥ 1.5	≥ 1.5	Positive

CSF INTERPRETATION OF RESULTS

Calculate CF EIA units by dividing the blanked OD value of each blue (CF) well by the blanked OD value of the blue (CF) Calibrator Cutoff well. Calculate TP EIA units by dividing the blanked OD value of each clear (TP) well by the blanked OD value of the clear (TP) Calibrator Cutoff well.

CF EIA Units	TP EIA Units	Interpretation
< 0.3	< 0.3	Negative
≥ 0.3	≥ 0.3	Positive

SAMPLE CALCULATIONS

$$\text{CF EIA Units} = \frac{\text{Blanked OD of Specimen (CF Well)}}{\text{Blanked OD of CF Calibrator Cutoff}}$$

$$\text{CF EIA Units} = \frac{0.426}{0.156} = 2.37 \text{ EIA Units}$$

$$\text{TP EIA Units} = \frac{\text{Blanked OD of Specimen (TP Well)}}{\text{Blanked OD of TP Calibrator Cutoff}}$$

$$\text{TP EIA Units} = \frac{1.125}{0.298} = 3.78 \text{ EIA Units}$$

LIMITATIONS OF THE PROCEDURE

The clarus *Coccidioides* EIA is intended for use with serum and CSF specimens only to aid in the diagnosis of coccidioidomycosis. The performance characteristics of this assay have not been evaluated for other types of specimens. All results should be reviewed considering other clinical data by the physician.

A negative result with both CF and TP tests does not preclude a diagnosis of coccidioidomycosis, particularly if only a single specimen has been tested and the patient shows symptoms consistent with a positive diagnosis. Diagnosis of coccidioidomycosis is based on laboratory and clinical findings.

Bloody CSF samples may test positive if patient has IgM or IgG antibodies towards *Coccidioides* in their blood.

All performance testing for this product was performed using manual operation. To set up this product for automated use, please contact the manufacturer of your automated EIA analyzer.

If 1X Wash Buffers appears cloudy or has sediment, please discard.

Positive results on either the IgM or IgG microwells suggest coccidioidomycosis. A patient with early-stage infection may present with a positive IgM result and negative on the IgG portion of the assay, whereas, a patient with a chronic or long-term infection may be positive on the IgG portion of the assay and negative on the IgM portion.

Do not test diluted patient specimens on other *Coccidioides* products. Specimen diluents are assay specific and the incorrect use may lead to erroneous results.

If excessive background is noted, increase wash volume and/or total number of washes.

The clarus *Coccidioides* EIA has two separate cutoffs for each matrix serum and CSF. Careful calculation and understanding are required to ensure the correct cutoff is used in patient diagnosis using this assay.

EXPECTED VALUES & PERFORMANCE CHARACTERISTICS

SERUM:

PERCENT AGREEMENT TO COMPLEMENT FIXATION/IMMUNODIFFUSION (N=1696)

The positive and negative percent agreement of the clarus *Coccidioides* Antibody EIA was evaluated versus *Coccidioides* complement fixation (CF). Discrepant results (EIA+/CF- and EIA-/CF+) were then tested using immunodiffusion (ID) for majority consensus. A total of 1696 serum samples were tested on the clarus *Coccidioides* Antibody EIA at a 1:441 dilution, using a cut-off value of 1.5 EIA Units.

Note: Positive results on either IgM and/or IgG plates indicates a positive result for the specimen.

Serum 1:441 Dilution	**CF/ID**		
IMMY clarus *Coccidioides* EIA	Positive	Negative	Total
Positive	289	86	390
Negative	34	1287	1306
Total	323	1373	1696

		95% CI
% Pos. Agreement	89.5%	86.6-92.6%
% Neg. Agreement	93.7%	92.3-95.0%

CSF:
SENSITIVITY AND SPECIFICITY TO EORTC/MSG CLINICAL CRITERIA (N=81)

The sensitivity and specificity of the clarus *Coccidioides* Antibody EIA were evaluated on patients classified as having proven coccidioidomycosis according to the EORTC/MSG consensus definitions.[8] A total of 81 CSF samples were tested on the clarus *Coccidioides* Antibody EIA at a 1:21 dilution, using a cut-off value of 0.3 EIA Units. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using this data.

Note: Positive results on either IgM and/or IgG plates indicates a positive result for the specimen.

CSF 1:21 Dilution	**EORTC**		
IMMY clarus *Coccidioides* EIA	Positive	Negative	Total
Positive	39	4	43
Negative	2	36	38
Total	41	40	81

		95% CI
Sensitivity	95.1%	83.5-99.4%
Specificity	90.0%	76.3-97.2%
PPV	90.7%	73.3-96.1%
NPV	94.7%	82.3-98.6%

HAZARD AND PRECAUTIONARY INFORMATION

Refer to the product Safety Data Sheets (SDS) for Hazards and Precautionary Statements.

SYMBOLS

	Contains sufficient for XX determinations		Catalogue Number/Reference Number
	Consult instructions for use		Batch/Lot Number
	Manufacturer		For In Vitro Diagnostic use
	Temperature Limitation		Control

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