Cocci Ab ELISA Kit

**Cat.No: DEIABL89**
**Lot. No. (See product label)**

**Size**

96T

**Intended Use**

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

**General Description**

Coccidioides species are dimorphic fungi that exist as either mycelia (saprobic growth) or spherules (parasitic growth) which cause respiratory diseases and occasionally diseases affecting other systems (1). Though endemic in the southwestern United States and Mexico, increased travel to the endemic areas has also increased the incidence in nonendemic areas (1,2). Coccidioidomycosis should be considered whenever patients display symptoms of pulmonary or meningeal infection and have lived or traveled to the endemic areas (3).

Coccidioidomycosis presents a diagnostic challenge to the physician and laboratorian. The manifestations of most early coccidioidal infections substantially overlap with those of other respiratory infections (4). In addition, culturally and histologically, the organisms can be difficult to demonstrate, even after repeated attempts (1, 2). 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 (1). 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 which 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 Coccidioides Antibody EIA is a sensitive, specific, and rapid test for the qualitative detection of TP and CF antibodies against antigens from Coccidioides.

**Principle Of The Test**

The Coccidioides Antibody EIA utilizes 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. Antibodies against TP antigens form early in the course of disease (typically IgM), followed by antibodies against CF (typically IgG) (1). Diluted patient specimens and controls are incubated in both TP and CF microwells. If anti-Coccidioides antibodies are present in patient specimens, the antibodies will become bound to the adsorbed antigens. Nonspecific reactants are removed by washing; peroxidase-conjugated, secondary anti-human antibody is then applied 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 washing. Substrate solution is then added to the microwells, developing color in the presence of peroxidase-conjugated secondary antibody. After adding stop solution, color change is quantified by measuring optical density (OD). Sample
OD readings are compared to calibrator cutoff OD readings to determine results.

**Reagents and Materials Provided**

A. TP Antigen-Coated Microwells (96) - Color-coded (clear = TP) stripwell plate featuring breakaway polystyrene microwells.
B. CF Antigen-Coated Microwells (96) - Color-coded (red = CF) stripwell plate featuring breakaway polystyrene microwells.
C. Positive Control (1.5 mL x 2) - Anti-Coccidioides antibodies in a buffered protein solution containing a preservative.
D. CF Calibrator Cutoff (1.5 mL x 2) - Anti-Coccidioides CF antibodies in a buffered protein solution (with a preservative) for establishing the cutoff signal for calculating CF EIA units.
E. TP Calibrator Cutoff (1.5 mL x 2) - Anti-Coccidioides TP antibodies in a buffered protein solution (with a preservative) for establishing the cutoff signal for calculating TP EIA units.
F. 10X Specimen Diluent (12 mL x 2) - Concentrated, buffered protein solution with a preservative.
G. 20X Wash Buffer (60 mL) - Concentrated wash buffer with a preservative.
H. TP Enzyme Conjugate (12 mL) - Affinity-purified rabbit anti-human IgM antibodies conjugated to horseradish peroxidase (HRP) in a buffered protein solution with a preservative.
I. CF Enzyme Conjugate (12 mL) - Affinity-purified rabbit anti-human IgG antibodies conjugated to horseradish peroxidase (HRP) in a buffered protein solution with a preservative.
J. TMB Substrate (12 mL x 2) - Buffered solution containing urea peroxide and tetramethylbenzidine.
K. Stop Solution (12 mL x 2) - 2 N sulfuric acid. CAUTION: AVOID CONTACT WITH SKIN. FLUSH WITH WATER IF CONTACT OCCURS.
L. Microwell Strip Holder (2)

**Materials Required But Not Supplied**

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 (A = 450 nm or dual absorbances at A = 450 nm and 630 nm)
E. Squirt bottle, EIA plate washer, or multi-channel pipettor for washing
F. Timer
G. Graduated cylinder for dilutions of wash buffer and specimen diluent

**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.

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.

Specimens should be brought to room temperature (22 to 25°C) prior to testing. Dilute serum 1:441 with 1X Specimen Diluent as follows:

A. 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.
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.

**Reagent Preparation**
REAGENT PREPARATIONS
A. The entire kit, including the microwell plates, should be at room temperature (22-25°C) for/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 Coccidioides Antibody ELISA 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 bags and sealed immediately after opening. Care should be taken to ensure the desiccant pouches remain in the bags with unused microwells. Store at 2-8°C.

Assay Procedure

CF wells – red
TP wells – clear
A. Bring all kit components to room temperature.
B. Snap off a sufficient number of wells (TP; CF) for samples and controls and insert them into the microwell holder. Record the position of each patient and the controls.
C. Dispense 100 μL of each diluted specimen into both red (CF) and clear (TP) wells.
D. Dispense 100 μL of Positive Control to a red (CF) well and 100 μL to a clear (TP) well. These wells will be the positive controls for the assay.
E. Dispense 100 μL of Specimen Diluent to a red (CF) well and 100 μL to a clear (TP) well. These wells will be the negative controls for the assay.
F. Dispense 100 μL of Specimen Diluent to a red (CF) well and 100 μL to a clear (TP) well. These wells will be the blanks for the assay.
G. Dispense 100 μL of CF Calibrator Cutoff to a red (CF) well and 100 μL of TP Calibrator Cutoff to a clear (TP) well. These wells will indicate the cutoff absorbances for the calculations of EIA units.
H. If running manually, gently shake 1-2 seconds to ensure reagent is at bottom of well (optional).
I. Incubate plate at room temperature (22-25°C) for 30 minutes.
J. Using a pipettor, aspirate the contents from the wells and discard into a biohazard receptacle.
K. Completely fill all wells with 1X Wash Buffer. This can be accomplished using a squirt bottle, EIA plate washer, or multichannel pipettor. If using a squirt bottle, direct the stream against the sides of the wells to avoid foaming. Dump the plate contents.
L. Repeat step K 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.
M. Dispense 100 μL of the CF Enzyme Conjugate to each of the red (CF) wells.
N. Dispense 100 μL of the TP Enzyme Conjugate to each of the clear (TP) wells.
O. If running manually, gently shake 1-2 seconds (optional). P. Incubate plate at room temperature (22-25°C) for 30 minutes.
Q. Repeat steps J-L.
R. Dispense 100 μL of TMB Substrate to each microwell. Start a timer with the addition of the substrate to the first well.
S. If running manually, gently shake 1-2 seconds to ensure reagent is at bottom of well (optional).
T. Incubate the plate at room temperature (22-25°C) for 10 minutes.
U. Dispense 100 μL of Stop Solution to each microwell in the same order as step R.
V. If running manually, gently shake 1-2 seconds to ensure reagent is at bottom of well (optional).
W. Read and record results (see READING THE TEST).

READING THE TEST
A. Reading the plate 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 blank wells (refer to Qualitative Screening Procedure, step D).
2. If a single wavelength reader is used, read the absorbance at 450 nm. Blank on blank wells (refer to Qualitative Screening Procedure, step D).
B. Calculate CF EIA units by dividing the absorbance value of each red (CF) well by the absorbance value of the red (CF) calibrator cutoff well. Calculate TP EIA units by dividing the absorbance value of each clear (TP) well by the absorbance value of the clear (TP) calibrator cutoff well.
C. Disinfect and retain microwell holder. Discard used assay materials as biohazard waste.

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 differentiations between negative and positive sera. Although the absorbance values may vary between runs and laboratories, the mean value for the CF calibrator cutoff must be within 0.100 to 0.250 OD units. The mean value for the TP calibrator cutoff must be within 0.200 to 0.350 OD units. Report results as index values (i.e. EIA units) relative to the calibrator cutoff. The positive control EIA units for both CF and TP should be between 2 and 6. The negative control EIA units should be less than 1. 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.

Calculation

SAMPLE CALCULATION

CF EIA Units = Blanked OD of Specimen (CF Well) / Blanked OD of CF Calibrator Cutoff

CF EIA Units = 0.426 / 0.156 = 2.73 EIA Units

TP EIA Units = Blanked OD of Specimen (TP Well) / Blanked OD of TP Calibrator Cutoff

TP EIA Units = 1.125 / 0.298 = 3.78 EIA Units

Interpretation of Results

Calculate CF EIA units by dividing the absorbance value of each red (CF) well by the absorbance value of the red (CF) Calibrator Cutoff well. Calculate TP EIA units by dividing the absorbance value of each clear (TP) well by the absorbance value of the clear (TP) Calibrator Cutoff well.
Performance Characteristics

RELATIVE SENSITIVITY AND SPECIFICITY TO COMPLEMENT FIXATION (N=716)
The relative sensitivity and specificity of the Coccidioides Antibody EIA were evaluated versus Coccidioides complement fixation (CF). A total of 716 specimens was tested using the Coccidioides Antibody EIA. The sample set consisted of 554 samples representing a normal population, 120 Coccidioides CF-positive samples with CF titers ranging from 1:2 through 1:256, and 42 samples from patients identified as positive for other fungal infections by immunodiffusion (ID) testing.

<table>
<thead>
<tr>
<th>Test</th>
<th>Positive</th>
<th>Negative</th>
<th>Total Pos.</th>
<th>Total Neg.</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>113</td>
<td>0</td>
<td>113</td>
<td>549</td>
<td>100%</td>
<td>99.6%</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>547</td>
<td>115</td>
<td>547</td>
<td>98.3%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Indeterminate results (Cocci-positive patients, n=5; Cocci-negative patients, n=7) were excluded from the sensitivity, specificity, PPV, and NPV calculations. Calculations were based on a comparison with a normal population; therefore, specimens exhibiting cross-reactivity for other fungal infections (n=42) were excluded from the table above. *Patients were classified as positive for Coccidioides antibody when the complement fixation (CF) test yielded a titer of 1:2 or greater.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
<th>Neg. *</th>
<th>Ind. **</th>
<th>Pos. ***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative Sensitivity with Coccidioides CF-positive patients (CF titer ≥ 1:2)</td>
<td>120</td>
<td>1.7% (2/120)</td>
<td>4.2% (5/120)</td>
<td>94.1% (113/120)</td>
</tr>
<tr>
<td>Relative Specificity with normal population</td>
<td>554</td>
<td>98.7% (547/554)</td>
<td>1.3% (7/554)</td>
<td>0% (0/554)</td>
</tr>
<tr>
<td>Cross-Reactivity with patients positive for other fungal infections</td>
<td>42</td>
<td>78.6% (33/42)</td>
<td>16.7% (7/42)</td>
<td>4.8% (2/42)</td>
</tr>
</tbody>
</table>

*Negative result indicates the specimen was negative for both CF and TP.
**Indeterminate result indicates the specimen was negative for either the CF or TP test and indeterminate for the other, or indeterminate for both CF & TP.
***Positive result indicates the specimen was positive for CF, TP, or both.
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 Coccidioides CF-positive specimens categorized according to titer. When the CF titer of the Coccidioides-positive specimen was greater than or equal to 1:4, which was the case in 88 of the 120 Coccidioides specimens, 95.4% of the specimens were identified as positive and the remaining 4.6% fell in the indeterminate range. None of the specimens with a CF titer of 1:4 or greater was identified as negative.

When the CF titer of the Coccidioides-positive specimen was greater than or equal to 1:8, which was the case in 69 of the 120 specimens, 100% of the samples were identified as positive. None of the specimens with a CF titer of 1:8 or greater was identified as negative or indeterminate.

*A negative result indicates the specimen was negative both for CF and TP.
** An Indeterminate result indicates that the specimen was negative for either the CF or TP test and indeterminate for either CF or TP or both.
***A positive result indicates that the specimen was positive for CF, TP, or both.

The high sensitivity and specificity of the Coccidioides Antibody EIA are achieved by utilizing separate antigen preparations for the detection of anti-CF and anti-TP antibodies. As a result, there is some variation in the sensitivities and specificities of the different antigen preparations. The data from the 716 specimens that were tested using the Coccidioides Antibody EIA was analyzed for sensitivity and specificity of the individual antigen preparations. A summary of this analysis is included in the table below.
**Sensitivity**

In a separate study, the relative sensitivity and specificity of the Coccidioides Antibody EIA were evaluated versus Coccidioides immunodiffusion (ID). A total of 85 Coccidioides ID-positive samples (IDCF identity band) was tested on the Coccidioides Antibody EIA following the package insert. Of the specimens that were ID-positive, 97.6% were found to be positive using the Coccidioides Antibody EIA. The remaining 2.4% of the specimens fell into the indeterminate range. None of the ID-positive specimens yielded a negative result in the EIA.

<table>
<thead>
<tr>
<th>CD Coccidioides ELISA Results</th>
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</thead>
<tbody>
<tr>
<td>Characteristic</td>
</tr>
<tr>
<td>Relative Sensitivity with</td>
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<tr>
<td>Coccidioides ID-positive patients</td>
</tr>
</tbody>
</table>

*Negative result indicates the specimen was negative for both CF and TP.
**Indeterminate result indicates the specimen was negative for either CF or TP and indeterminate for the other, or indeterminate on both CF and TP.
***Positive result indicates the specimen was positive for CF, TP, or both.

**Precautions**

**REAGENT PRECAUTIONS**

A. All reagents are intended for in vitro use only!
B. Specific standardization is necessary to produce our high-quality reagents and materials. CD 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.
Limitations

LIMITATIONS OF THE PROCEDURE
The Coccidioides EIA is intended for use with serum 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 in light of 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.

References