sōna *Coccidioides* Ab LFA Test Kit

For the Detection of Coccidioides Antibodies – REF CTA2003
For *in vitro* diagnostic use only.

IVD 2°C – 25°C

INTENDED USE

The sōna *Coccidioides* Antibody Lateral Flow Assay (LFA) 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).

EXPLANATION

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 enzyme immunoassay (EIA) are 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 EIA assay is sensitive and specific but requires additional laboratory equipment. However, the sōna *Coccidioides* Antibody LFA is a sensitive, specific, and rapid test for the qualitative detection of IgM and IgG antibodies directed against TP and CF antigens from Coccidioides species.

BIOLOGICAL PRINCIPLES

The sōna *Coccidioides* Antibody LFA utilizes a mixture of modified and native *Coccidioides* antigens, including the CF and TP antigens, adsorbed to nitrocellulose. Antibodies against TP antigens form early in the course of disease (typically IgM), followed by antibodies against CF (typically IgG)[1]. The assay does not distinguish between IgG and IgM. Diluted patient specimens are applied to the LFA strips. If anti-*Coccidioides* antibodies are present in patient specimens, the antibodies will become bound to the adsorbed antigens. If patient antibodies are bound to the adsorbed antigens, the gold-conjugated antibody-binding proteins will become bound to the patient antibodies and result in the formation of a red test line (positive) and control line. If patient antibodies are not bound to the adsorbed antigens, the gold conjugate will bind only to the control line (negative).

REAGENTS

A. *Coccidioides* Ab Lateral Flow Test Strips (REF LFCA50) (50 strips) – Strips packaged in a desiccant tube.
B. Specimen Diluent (REF LFASD1) (25 mL) –Buffered protein solution with a preservative.
C. *Coccidioides* Ab Positive Control (REF CTAPC1) (1 mL) – Mixture of anti-*Coccidioides* antibodies in a buffered protein solution with a preservative.

REAGENT PRECAUTIONS

A. All reagents are intended for in vitro diagnostic use (IVD).
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. Use only protocols described in this package insert. Incubation times or temperatures other than those specified may give erroneous results. 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 splashing when dispensing reagents into the tubes or plate wells as this can cause erroneous results.
F. Use fresh disposable pipette tips when appropriate to avoid contamination of results.

REAGENT STABILITY AND STORAGE

The entire sōna *Coccidioides* Antibody LFA kit should be stored at 2-25 °C until the expiration dates listed on the kit label.

Unused LFA strips should be kept in the desiccant tube, and the desiccant tube should remain sealed when not in use.

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.

Serum

Dilute serum **1:441** with Specimen Diluent as follows:
A. Obtain 2 tubes for each serum specimen. Transfer 200 µL of Specimen Diluent to the first tube and 200 µL to the second tube.
B. Mix the specimen thoroughly.
C. Transfer 10 µL of serum to the first tube and mix thoroughly.
D. Transfer 10 µL of the first dilution into the second tube and mix thoroughly (**1:441** dilution).

CSF

Dilute CSF **1:21** with Specimen Diluent as follows:
A. Obtain 1 tubes for each CSF specimen. Transfer 200 µL of Specimen Diluent to this tube.
B. Mix the specimen thoroughly.
C. Transfer 10 µL of CSF to the first tube and mix thoroughly (**1:21** dilution).

PROCEDURE

REFER TO REAGENTS SECTION FOR A LISTING OF MATERIALS PROVIDED.

MATERIALS NOT PROVIDED

A. Pipette(s) capable of measuring and delivering 10 µL, 100 µL, 200 µL, and appropriate disposable tips.
B. Tubes for dilution of specimens.
C. Flat-bottom tubes or 96-well assay plate (untreated) for running test.
D. Timer.

PROCEDURE

Serum

A. If not stored at room temperature, bring kit to room temperature.
B. Dispense 100µL of each 1:441 diluted serum specimen into separate flat-bottom tubes or plate wells. Be sure that all the specimen is in the bottom of the tube.
C. Insert strip into tube or well within 10 minutes (↓↓ down).
D. Incubate at room temperature (20-25 °C) for 30-60 minutes.
E. Read and record results (see READING THE TEST).

CSF

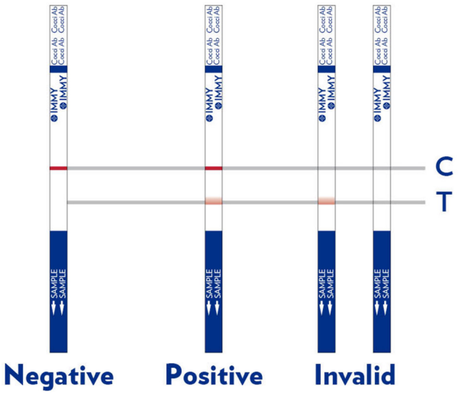
A. If not stored at room temperature, bring kit to room temperature.
B. Dispense 100 µL of each 1:21 diluted CSF specimen into separate flat-bottom tubes or plate wells. Be sure that all the specimen is in the bottom of the tube.
C. Insert strip into tube or well within 10 minutes (↓↓ down).
D. Incubate at room temperature (20-25 °C) for 30-60 minutes.
E. Read and record results (see READING THE TEST).

QUALITY CONTROL PROCEDURE

A. It is recommended to perform the quality control procedure with every new shipment or lot received.
B. Add 3 drops (120 µL) of undiluted Coccidioides Ab Positive Control (REF CTAPC1) into a tube or plate well.
C. Pipette 100 µL of Specimen Diluent (REF LFASD1) into a separate tube or plate well.
D. Insert strip into tubes or wells (↓↓ down).
E. Incubate at room temperature (20-25 °C) for 30-60 minutes.
F. Read and record results (see READING THE TEST).

READING THE TEST

A. Ensure entire reagent/sample has been fully absorbed from the tube or well onto the test strip at the end of incubation.
B. The presence of a control line only (C=Control; see diagram) is a negative result.
C. The presence of 2 pink or red lines (C=Control and T=Test) is a positive result. The width of the test line may vary. Note: A gray test line should not be considered positive. Holding the strip against a white background may assist in the distinction of a test line.
D. The test must be read within 60 minutes of incubation. Reading after this window of time may provide erroneous results.



QUALITY CONTROL

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

Control Line must be present for valid results.

LIMITATIONS OF THE PROCEDURE

The sōna *Coccidioides* Ab LFA 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 in light of other clinical data by the physician.

A negative result test 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.

INTERFERENCE

This assay was evaluated for the potential of interference due to serum conditions including icteric, hemolyzed, and lipemic samples. Cerebrospinal Fluid conditions related to high levels of bilirubin, blood, protein, and iodine were also evaluated. These samples exhibited no interference in the assay.

CROSS REACTIVITY ANALYSIS

The sōna *Coccidioides* Ab LFA was evaluated for cross-reactivity against a panel of patients' serum specimens across a variety of pathologies. The results of this testing are shown in the table below.

Pathology	# of Samples	% Positive
Mycoplasmosis	5	20% (1/5)
HIV+	5	0% (0/5)
ANA +	4	0% (0/4)
Blastomycosis	2	0% (0/2)
Cryptococcosis	4	0% (0/4)
Histoplasmosis	5	80% (4/5)
Aspergillus Ab+	5	0% (0/5)
Rh+	4	0% (0/4)

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This assay was not evaluated for cross-reactivity against the following organisms or pathologies:

Candida dubliniensis
Candida tropicalis
Candida parapsidosis
Candida krusei
Candida glabrata
Cladosporium trichoides
Neisseria meningitidis
Salmonella typhi
Mycobacterium tuberculosis
Enterovirus
Enterobacteriaceae
Enterococcus spp.
Epstein Barr
Pneumocystis carinii
Trichosporon beigelii
Zygomycetes
Staphylococcus aureus
Hepatitis A Virus
Hepatitis C Virus
Staphylococcus spp.
Streptococcus pneumonia
Streptococcus spp.
Diphtheroid
H. influenzae type B
Herpes simplex viruses
Listeria monocytogenes
Syneresis fluid condensation

Cross-reactivity in CSF samples was not evaluated.

SPECIFIC PERFORMANCE CHARACTERISTICS

SERUM

Immunodiffusion Method Comparison

The sōna *Coccidioides* Ab LFA was compared to *Coccidioides* immunodiffusion (ID) performed at a reference laboratory to evaluate the percent agreement in serum samples. The results can be found in the tables below Note: All specimens were sent to the reference laboratory because physicians suspected a *Coccidioides* infection.

	Immunodiffusion			
	IgG & IgM Positive	IgG Positive only	IgM Positive Only	IgG & IgM Negative
CTA2003 Pos	15	102	24	5
CTA2003 Neg	0	1	1	57

	Immunodiffusion Overall	
	Positive	Negative
CTA2003 Pos	141	5
CTA2003 Neg	2	57

	Point Estimate	95% CI
Percent Agreement Positive	98.6%	95.0% - 99.8%
Percent Agreement Negative	91.9%	82.2% - 97.3%
Positive Likelihood Ratio	12.23	5.27 - 28.34
Negative Likelihood Ratio	0.02	0.00 - 0.06
Positive Predictive Value	96.6%	92.2% - 98.9%
Negative Predictive Value	96.6%	88.3% - 99.5%

Enzyme Immuno Assay (EIA) Method Comparison

The sōna *Coccidioides* Ab LFA was compared to a commercially-available Coccidioidomycosis Enzyme Immunoassay (EIA) on samples submitted to a reference laboratory to evaluate the percent agreement in serum samples. The results can be found in the tables below. Note: Indeterminates were removed from the data for point estimate calculations.

	EIA		
	Positive	Indeterminate	Negative
CTA2003 Pos	139	0	7*
CTA2003 Neg	3	2	54

	Point Estimate	95% CI
Percent Agreement Positive	97.9%	93.9% – 99.5%
Percent Agreement Negative	88.5%	77.7% - 95.2%
Positive Predictive Value	95.2%	90.4% - 98.0%
Negative Predictive Value	94.7%	85.4% - 98.8%

** Two samples were ID and/or CF positive*

Complement Fixation (CF) Method Comparison

The sōna *Coccidioides* Ab LFA was compared to *Coccidioides* complement fixation (CF) performed at reference laboratory to evaluate the percent agreement in serum samples. The results can be found in the tables below. Note: All specimens were sent to the reference laboratory because physicians suspected a *Coccidioides* infection.

	Complement Fixation	
	Positive	Negative
CTA2003 Pos	91	5
CTA2003 Neg	2	56

	Point Estimate	95% CI
Percent Agreement Positive	97.9%	92.4% - 99.7%
Percent Agreement Negative	91.8%	81.9 - 97.3%
Positive Predictive Value	94.8 %	88.7 - 97.7%
Negative Predictive Value	96.6%	87.6 - 99.1%

In the same study, a total of 37 Complement Fixation Negative samples were determined to be positive for anti-*coccidioides* antibodies because they were positive on IMMY's *Coccidioides* Antibody EIA (REF# CAB102), another commercially available *Coccidioides* Antibody EIA, and an immunodiffusion assay. These 37 samples were also positive on CTA2003. These samples were excluded from the analysis above.

Specificity Performance

The sōna *Coccidioides* Ab LFA specificity was evaluated using healthy blood donor serum specimens from an endemic region (Arizona n=121) and a non-endemic region (Puerto Rico n=45). These specimens are described by Lindsley et. al. (5). Summary tables of the data collected are included below.

	Presumed Negative
	Negative
CTA2003 Pos	6
CTA2003 Neg	160

	Point Estimate	95% CI
Specificity	96.4%	92.3% - 98.7%

** All 6 positives are from the endemic region. No samples from the non-endemic region were positive on the LFA.*

CEREBROSPINAL FLUID (CSF)

EORTC Method Comparison

The sōna *Coccidioides* Ab LFA was evaluated at a 1:21 dilution using EORTC defined CSF specimens and compared against true patient status (EORTC)[6].

	Coccidioidal Meningitis	
	Positive	Negative
CTA2003 Pos	40	0
CTA2003 Neg	2	12

	Point Estimate	95% CI
Sensitivity	95.2%	83.8% - 99.3%
Specificity	100%	73.4% - 100%
Positive Predictive Value	100%	91.1% - 100%
Negative Predictive Value	85.7%	57.2% - 97.8%

REPRODUCIBILITY

The sōna *Coccidioides* Ab LFA was evaluated for reproducibility and precision by testing three positive and two negative serum specimens. Positive specimens tested ranged from strong to very weak positive, based on IMMY's *Coccidioides* Antibody EIA (REF CAB102). This panel was tested in triplicate, daily for five days and read by one operator. The results of the study are shown in the following table.

SERUM

	Overall % Pos
High Positive	100% (15/15)
Low Positive	100% (15/15)
Low Positive	100% (15/15)
Neg	0% (0/15)
Neg	0% (0/15)

CSF

	Overall % Pos
High Positive	100% (15/15)
Low Positive	100% (15/15)
Low Positive	100% (15/15)
Neg	0% (0/15)
Neg	0% (0/15)

BIBLIOGRAPHY

1. Pappagianis, D. and B.L. Zimmer. 1990. Serology of coccidioidomycosis. Clin. Microbiol. Reviews, 3:247-268
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5. Lindsley, M.D., Y. Ahn, O. McCotter, L. Gade, S.F. Hurst, M.E. Brandt, B.J. Park, and A.P. Litvintseva. 2015. Clin Vaccine Immunol., 22 (10): 1090-5.
6. Stevens, D.A., Martinez, M., Sass, G., Pappagianis, D., Doherty, B., Kutsche, H., McGuire, M. Comparative Study of Newer and Established Methods of Diagnosing Coccidioidal Meningitis. 2020. Journal of Fungi, 6, 125.

INTERNATIONAL SYMBOL USAGE

2°C 25°C	Storage 2-25°C	LOT	Lot Number
	Manufactured by	REF	Reference Number
	Expiration Date	IVD	In Vitro Diagnostics
	Protect from Humidity	Σ	Sufficient for "#" Tests

IMMY, Inc.
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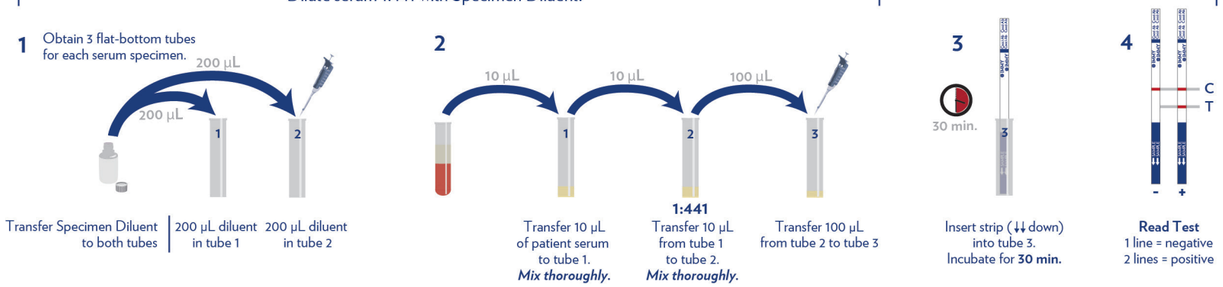
SERUM PROCEDURE – 1:441 dilution

SPECIMEN PREPARATION — Dilute serum 1:441 with Specimen Diluent:

RUN TEST



CSF PROCEDURE – 1:21 dilution

SPECIMEN PREPARATION — Dilute CSF 1:21 with Specimen Diluent:

RUN TEST