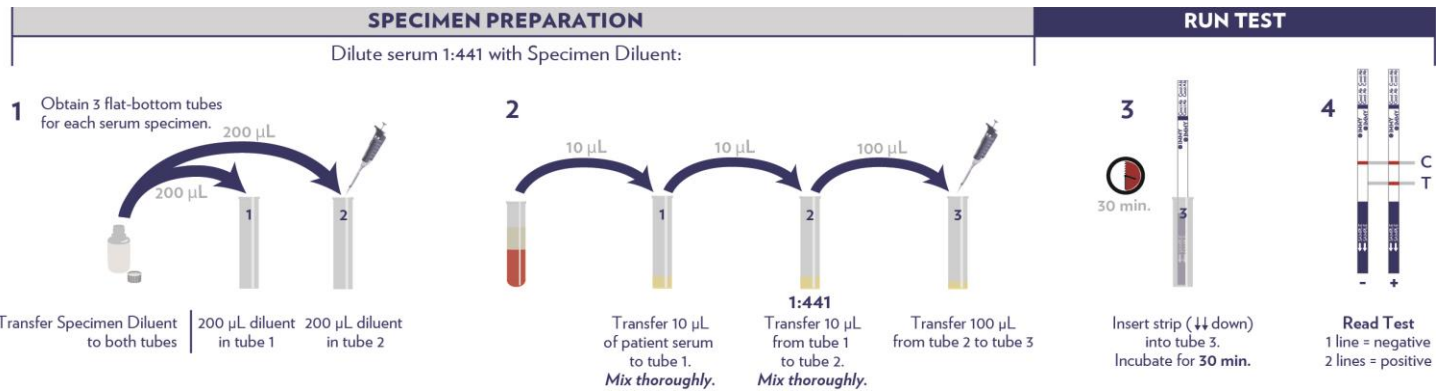


For *in vitro* diagnostic use only.



INTENDED USE

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

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 antibodies against the TP and CF antigens from *Coccidioides*.

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). 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

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

REAGENT PRECAUTIONS

- All reagents are intended for *in vitro* diagnostic use (IVD).
- 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.
- 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.
- 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.
- Avoid splashing when dispensing reagents into the tubes or plate wells as this can cause erroneous results.
- 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.

Dilute serum 1:441 with Specimen Diluent as follows:

- Obtain 2 tubes for each serum specimen. Transfer 200 μ L of Specimen Diluent to the first tube and 200 μ L to the second tube.
- Mix the specimen thoroughly.
- Transfer 10 μ L of serum to the first tube and mix thoroughly.
- Transfer 10 μ L of the first dilution into the second tube and mix thoroughly (1:441 dilution).

PROCEDURE

REFER TO REAGENTS SECTION FOR A LISTING OF MATERIALS PROVIDED.

MATERIALS NOT PROVIDED

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

PROCEDURE

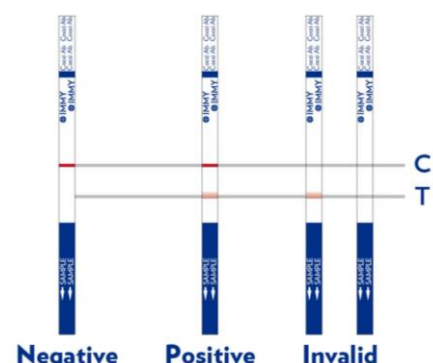
- If not stored at room temperature, bring kit to room temperature.
- Dispense 100 μ L of each 1:441 diluted specimen into separate flat-bottom tubes or plate wells. Be sure that all the specimen is in the bottom of the tube.
- Insert strip into tube or well (⇓ down).
- Incubate at room temperature (20-25 °C) for 30-60 minutes.
- Read and record results (see READING THE TEST). *Note: This test must be ran in a timely manner to avoid tubes or wells drying out.*

QUALITY CONTROL PROCEDURE

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

READING THE TEST

- The presence of a control line only (C=Control; see diagram) is a negative result.
- 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.*
- 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 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 (n=15). 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' 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)

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*
- Syneresis fluid condensation
- Pneumocystis carinii*
- Trichosporon beigelii*
- Zygomycetes*
- Staphylococcus aureus*
- Hepatitis A Virus
- Hepatitis C Virus
- Staphylococcus* spp.
- Streptococcus pneumoniae*
- Streptococcus* spp.
- Diphtheroid*
- H. influenzae* type B
- Herpes simplex viruses*
- Listeria monocytogenes*

SPECIFIC PERFORMANCE CHARACTERISTICS

Immunodiffusion Method Comparison

The **sōna** *Coccidioides* Ab LFA was compared to *Coccidioides* immunodiffusion (ID) performed at a reference laboratory to evaluate the percent agreement. *Note: All specimens were sent to the reference laboratory because physicians suspected a Coccidioides infection. Summary tables of the data collected are below.*

	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. 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

** One sample was IgM positive on ID; one sample was negative on CF and ID; one sample was weak CF positive and ID negative.

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. *Note: All specimens were sent to the reference laboratory because physicians suspected a Coccidioides infection. Summary tables of the data collected are included below.*

	Complement Fixation
	Positive
CTA2003 Pos	91
CTA2003 Neg	2

	Point Estimate	95% CI
Percent Agreement Positive	97.9%	92.4% - 99.7%
Positive Predictive Value	100.0%	96.0% - 100%

Specificity Performance

The **sōna** *Coccidioides* Ab LFA specificity was evaluated using healthy blood donor specimens from an endemic region (Arizona n=124) and a non-endemic region (Puerto Rico n=37). These specimens are described by Lindsley *et. al.* (5). Summary tables of the data collected are included below.

	Presumed Negative
	Negative
CTA2003 Pos	10*
CTA2003 Neg	151

	Point Estimate	95% CI
Specificity	93.8%	88.9% - 97.0%
Negative Predictive Value	100%	97.6% - 100%

* All 10 positives are from the endemic region. No samples from the non-endemic region were positive on the LFA. Four of the 10 LFA positives are EIA positive.

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 table below.

	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)

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