LA-Coccidioides, LA-Histoplasma, and LA-Sporothrix Antibody Systems

REF CL1001, HL1001, SL1001, and Individual Reagents

IVD

INTENDED USE

The LA-*Coccidioides* (REF CL1001), LA-*Histoplasma* (REF HL1001), and LA-*Sporothrix* (REF SL 1001) antibody detection systems are semi-quantitative tests that detect agglutinating antibodies against *Coccidioides immitis (posadasii), Histoplasma capsulatum,* or *Sporothrix schenckii* in the serum of patients with coccidioidomycosis, histoplasmosis, or sporotrichosis, respectively, to aid in the diagnosis of disease.

EXPLANATION

Coccidioidomycosis

C. immitis (California isolates) and *C. posadasii* (non-California isolates) are somewhat localized in the southwestern United States (5) and Central and South America, but modern transportation has increased the likelihood of infection for individuals visiting the region. The LA-*Coccidioides* test is a sensitive and rapid screening test, but it has a false-positive rate, so confirmation of the results of latex agglutination (LA) by immunodiffusion (ID) and/or complement fixation (CF) is recommended (4).

Histoplasmosis

Histoplasmosis results from infection with *H. capsulatum,* which has a worldwide distribution, but is a particular problem in central and southeastern areas of the United States and in certain regions of Central and South America (16) The LA-*Histoplasma* test is useful for the presumptive early detection of acute histoplasmosis (12). A positive LA test result can be demonstrated as early as 2 to 3 weeks after infection with *H. capsulatum*; however confirmation of the LA test result by ID and/or CF is recommended (12).

Sporotrichosis

Sporotrichosis is an endemic fungal infection caused by *S. schenckii* with most case reports coming from the tropical and subtropical regions of the Americas (13). The LA-*Sporothrix* test is a sensitive, rapid test that is useful for the presumptive diagnosis of sporotrichosis from patients with localized cutaneous, subcutaneous, disseminated subcutaneous or systemic forms of the disease (9).

BIOLOGICAL PRINCIPLES

The LA-*Coccidioides*, LA-*Histoplasma*, and LA-*Sporothrix* antibody systems are based upon the principle that *Coccidioides*, *Histoplasma*, or *Sporothrix* antigen-coated latex particles will agglutinate with specimens containing anti-*Coccidioides*, anti-*Histoplasma*, or anti-*Sporothrix* antibodies, respectively. The LA-tests detect agglutinating antibodies that are of sufficiently large size to bridge between antigen-coated latex particles (10). Immunoglobulins other than IgM (e.g. IgG, IgA, and IgD) are not as effective at bridging between the sensitized particles, and therefore positive reactions by LA are mostly associated with IgM antibodies. (10).

MATERIALS PROVIDED

LA-*Coccidioides* Antibody System

A. ***Coccidioides*** **Latex** (4 ml, **REF** CX0000): Contains a standardized suspension of *Coccidioides* antigen-coated latex particles and a preservative.
B. ***Coccidioides*** **Latex Positive Control** (1 ml, **REF** CE0000): Contains serum from goats immunized with C. *immitis* antigens and a preservative.
C. **Negative Control** (1 ml, **REF** N80110): Normal goat serum containing a preservative.
D. **Disposable Ring Slides** (**REF** SC0020)

LA-*Histoplasma* Antibody System

A. **Specimen Diluent** (10 ml, **REF** GB0020): Concentrated (10X) glycine buffered saline (pH 8.6) containing albumin and a preservative.
B. ***Histoplasma*** **Latex** (4 ml, **REF** HB0000): Contains a standardized suspension of *Histoplasma* antigen-coated latex particles and a preservative.
C. ***Histoplasma*** **Latex Positive Control** (1 ml, **REF** HC0000): Contains serum from goats immunized with *H. capsulatum* antigens and a preservative.
D. **Negative Control** (1 ml, **REF** N80110): Normal goat serum containing a preservative.
E. **Disposable Ring Slides** (**REF** SC0020)

LA-*Sporothrix* Antibody Detection System

A. **Specimen Diluent** (10 ml, **REF** GB0020): Concentrated (10X) glycine buffered saline (pH 8.6) containing albumin and a preservative.
B. ***Sporothrix*** **Latex** (4 ml, **REF** SX0000): Contains a standardized suspension of *Sporothrix* antigen-coated latex particles and a preservative.
C. ***Sporothrix*** **Latex Positive Control** (1 ml, **REF** S20000): Contains lyophilized serum from rabbits immunized with *S. schenckii* antigens and a preservative.
D. **Negative Control** (1 ml, **REF** N80110): Normal goat serum containing a preservative.
E. **Disposable Ring Slides** (**REF** SC0020)

MATERIALS NOT PROVIDED

A. Distilled or DI water
B. 1-ml serological pipettes
C. Disposable borosilicate glass test tubes (non-siliconized), 10 or 12 X 75 mm, for specimen dilutions
D. Test tube rack
E. Water bath or heat block (56° C)
F. Wooden applicator sticks
G. Rotator set to 100 rpm
H. Timer
I. Pipettor (25 µL and 100 µL)

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.
C. Do not use reagents containing foreign matter, particulates or aggregates, which indicate contamination or improper storage or handling.
D. Specimens must not contain bacteria, visible lipids, or other obvious signs of contamination.
E. Do not store specimens in a frost-free freezer. Repeated freezing and thawing of the specimens can affect test results.
F. When handling patient specimens, adequate measures should be taken to prevent exposure to etiologic agents potentially present in the specimen.
G. All reagents are preserved with sodium azide [0.095% (w/w)], which is a skin irritant. It is recommended that excess reagents be discarded in an appropriate waste receptacle.

STABILITY AND STORAGE

All reagents should be stored at 2-8° C, and are stable until the expiration date. Prolonged periods at room temperature should be avoided. Avoid **FREEZING** latex suspensions, as this causes granularity, which might be interpreted as a false positive reaction.

REAGENT PREPARATION

A. Specimen diluent (**REF** GB0020) should be diluted 1:10 with distilled or DI water prior to use.
B. Latex positive controls (**REF** CE0000, HC0000, and S20000) are rehydrated by adding 1 ml of distilled or DI water to the vial and incubating at room temperature until completely dissolved followed by gentle mixing.
C. When using the *Coccidioides* and *Histoplasma* latex positive controls (**REF** CE0000, HC0000) and negative control (**REF** N80110) for the first time, heat inactivate at 56° C for 30 minutes.
NOTE: When using the LA-*Sporothrix* antibody detection system (**REF** SL1001), ***DO NOT*** heat treat the *Sporothrix* positive control (**REF** S20000) or negative control (**REF** N80110).
D. Latex suspensions (**REF** CX0000, HB0000, and SX0000) must appear as homogeneous suspensions – MIX WELL prior to each use!
E. The ring slides (**REF** SC0020) should be discarded after each use.

SPECIMEN PREPARATION

A. Collect whole blood aseptically following accepted procedures. The specimen must not contain anticoagulants as this will invalidate the test.
B. Permit blood to clot for 10 minutes or more at room temperature in a collection tube.
C. Centrifuge 1000 x g for 15 minutes.
D. Carefully aspirate the serum into a sterile container and seal.
E. Specimen may be processed immediately, refrigerated, preserved by freezing at -20° C, or by adding thimerosal to provide a final concentration of 0.01%.
F. Incubate specimen at 56° C for 30 minutes.

COMPLETE THE FOLLOWING STEPS FOR LA-*HISTOPLASMA* and LA-*SPOROTHRIX ONLY*
Complete the following dilutions for LA-*Histoplasma* and LA-*Sporothrix* ONLY

A. Add 100 µl of specimen diluent (**REF** GB0020) to each of 6 tubes labeled 1-6, and place in a rack (1:2 through 1:64 dilutions). Additional dilutions may be necessary if the specimen is positive at 1:64.
B. Add 100 µl of patient specimen to tube #1 and mix well.
C. Transfer 100 µl from tube #1 to tube #2 and mix well. Continue this dilution procedure through tube #6.
D. Specimen dilutions are ready for testing (see PROCEDURE).

NOTE: Diluted specimens are **NOT** recommended for LA-*Coccidioides* (11, 12).

PROCEDURE

A. Add 25 µl of latex positive control (**REF** CE0000, HC0000, or S20000), negative control (**REF** N80110), and each specimen dilution (or undiluted specimen for LA-*Coccidioides*) onto separate rings of the ring slide.
B. Add one (1) drop of *Coccidioides*, *Histoplasma*, or *Sporothrix* latex (**REF** CX0000, HB0000, SX0000, respectively) onto each ring.
C. Using separate applicator sticks, thoroughly mix the contents of each ring.
D. Rotate by hand or place the ring slide on a rotator set to 100 rpm (+/-25) for 10 minutes at room temperature.
E. Read the reactions immediately (see **Reading the Test**).

Reading the Test

Read the reactions immediately over a dark background, and rate them on a scale from negative to 4+. Do not magnify. For comparison, the latex positive control should give a 2+ or greater reaction, and the negative control should be less than 1+ reaction. The graduations of the reaction strengths are as follows:

Negative (-): A homogeneous suspension of particles with no visible clumping.
One Plus (1+): Fine granulation against a milky background.
Two Plus (2+): Small but definite clumps against a slightly cloudy background.
Three Plus (3+): Large and small clumps against a clear background.
Four Plus (4+): Large clumps against a very clear background.

QUALITY CONTROL

Latex Control

Periodically, the sensitivity of the latex reagent (**REF** CX0000, HB0000, SX0000) may be tested by titering their respective positive controls (**REF** CE0000, HC0000, S20000). The positive control should be 2+ at 1:4 ±1 dilutions if the sensitivity of the latex reagents is satisfactory.

INTERPRETATIONS OF RESULTS

Control Reactions

The latex positive control must be 2+ or greater and the negative control must be less than 1+. If either control is incorrect one or both of the reagents is unsatisfactory (or the tests were performed improperly) and any patient tests with the reagents are invalid. A positive reaction with the negative control may indicate possible contamination or freezing of the latex, which could produce false positive results in patient specimens.

Patient Specimens

A. Coccidioidomycosis: A positive LA reaction with undiluted sera should be confirmed by ID or CF testing (12). A 2+ or greater reaction is considered a positive result.
B. Histoplasmosis: LA test titers of 1:16 or greater are considered presumptive evidence of active or very recent infection by *H. capsulatum* (12). The titer is the highest serum dilution that gives a 2+ agglutination (12).

LIMITATIONS OF THE PROCEDURE

Coccidioidomycosis

False positives can occur, so confirmation of the results by immunodiffusion (ID) and/or complement fixation (CF) is recommended (4). False negative reactions may occur in immunocompromised or immunosuppressed patients (1-3). No single test is adequate for detecting all positive specimens from cases of coccidioidomycosis (7, 15).

Histoplasmosis

Cross reactions with other systemic mycoses may occur with the LA test (12). Results should be interpreted with caution, particularly if only one specimen has been examined and the titer is low (12). The test should be repeated after 1 to 3 months (12). Additionally, confirmation of the LA test result by ID and/or CF is recommended (12). False negative reactions may occur in immunocompromised or immunosuppressed patients or persons with chronic histoplasmosis (12). Agglutinating antibodies to *H. capsulatum* antigens may be significantly increased in histoplasmin skin testing (12).

Sporotrichosis

False positive reactions have been noted at titers of 1:8 with sera from patients with non-fungal infections (12).

EXPECTED VALUES AND SPECIFIC PERFORMANCE CHARACTERISTICS

Coccidioidomycosis

The LA test is not as specific as the immunodiffusion IDTP test; at least 6% false positive reactions may occur with the LA test (7, 9). The LA and ID tests performed simultaneously detected 93% of the coccidioidomycosis cases (8). The LA test has a sensitivity and specificity of 66% and 93%, respectively (7).

Histoplasmosis

A positive LA test result can be demonstrated as early as 2 to 3 weeks after infection with *H. capsulatum*; therefore the LA test is an excellent presumptive test that aids in the diagnosis of acute histoplasmosis (12). The LA test has sensitivity of 100% and 46% for acute primary pulmonary and chronic pulmonary infections, respectively, and an overall sensitivity of 62% (6). The specificity of the LA test is 97% (6).

Sporotrichosis

Sera from patients with localized cutaneous lymphocutaneous or extracutaneous sporotrichosis may show titers that range from 1:8 to 1:512 (12). An increasing titer or a sustained high titer is helpful in the diagnosis of pulmonary sporotrichosis (12). The LA test has limited prognostic value (12). The LA test has a sensitivity of 100%, 86%, 73%, and 56% for disseminated, articular, pulmonary, and cutaneous disease, respectively (14). The specificity of the LA test is 100% (14).

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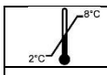
INTERNATIONAL SYMBOL OF USAGE

	Storage 2-8 °C	LOT	Lot Number
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