Histoplasma DxSelect™

Enzyme-linked Immunosorbent Assay (ELISA)
Product Code EL1700

Rev. E

Enzyme-linked immunosorbent assay for the qualitative detection of human antibodies to *Histoplasma capsulatum* var. *capsulatum*

Within the U.S.: For *in vitro* diagnostic use.
Outside the U.S.: For research use only.

INTENDED USE

The Focus Diagnostics Histoplasma DxSelect™ is intended for qualitatively detecting antibodies to *Histoplasma capsulatum* var. *capsulatum* in human serum. The test is indicated for testing persons having symptoms of respiratory disease, as an aid in the presumptive laboratory diagnosis of histoplasma infection. This test is not intended for self-testing, and this test is neither FDA cleared nor approved for testing blood or plasma donors. Assay performance characteristics have not been established for automated instruments.

SUMMARY AND EXPLANATION OF TEST

Histoplasma capsulatum is a fungus that causes respiratory diseases and occasionally diseases affecting other systems.[1] Most infected persons have no apparent ill effects. If symptoms occur, they start within 3 to 17 days after exposure.[1] The acute respiratory disease is characterized by respiratory symptoms, a general ill feeling, fever, chest pains, and a dry or non-productive cough. Distinct patterns may be seen on a chest x-ray. Chronic lung disease resembles tuberculosis and can worsen over months or years. The disseminated form is fatal unless treated.[1] Risk groups include those in endemic area with occupational exposure to bird or bat droppings (e.g., construction or agricultural workers, spelunkers).[2] High risk groups include immunocompromised persons (e.g., persons with cancer, transplant recipients, persons with HIV infection).[2]

In North America, there are several fungal pathogens that cause respiratory disease. *Histoplasma capsulatum* and *Blastomyces dermatitidis* are endemic in the Ohio and Mississippi river valleys, while *Coccidioides immitis* is endemic in the southwestern U.S. and northwestern Mexico.

Histoplasma capsulatum grows in soil and material contaminated with bat or bird waste. Spores become airborne when contaminated soil is disturbed. Breathing the spores causes infection. The disease is not transmitted from an infected person to another person.

Histoplasmosis is not a nationally reportable disease, but is reportable in some states. Where reportable, incidence is about 1 reported case per 100,000 population. Another variety of Histoplasma exists in Africa *Histoplasma capsulatum* var. *duboisii*.[2]

Complement fixation (CF) and immunodiffusion (ID) traditionally have been the methods most commonly used to detect fungal antibodies.[3] CF assays are sensitive, however their performance is complex and labor-intensive, and they exhibit low specificity due to cross-reactive antibodies recognizing carbohydrate moieties common to several fungi. ID is more specific, but less sensitive than CF. ID takes 48 hours to perform and requires highly skilled personnel to properly interpret results. Recently an ELISA-based method to identify *H. capsulatum* antibody was found to be a valuable adjunct to histoplasma serodiagnosis.[4]

TEST PRINCIPLE

In the Focus Diagnostics Histoplasma DxSelect™ assay, the polystyrene microwells are coated with inactivated histoplasma antigen. Diluted serum samples and controls are incubated in the wells to allow anti- histoplasma antibodies (if present in the sample) to react with the antigen. Nonspecific reactants are removed by washing and peroxidase-conjugated anti-human antibody is added that reacts with human antibodies bound to the antigen. Excess conjugate is removed by washing. Enzyme substrate and chromogen are added, and the color is allowed to develop. After adding the Stop Reagent, the resultant color change is quantified by a spectrophotometric reading of optical density (OD). Sample optical density readings are compared with reference cut-off OD readings to determine results.

MATERIALS SUPPLIED

Antigen Wells, 96 wells | REF | EL1701 | Ag |

12 eight-well polystyrene microwell strips on a frame. Each well is coated with inactivated histoplasma antigen. Each strip may be broken down into individual wells for cost effective use. To avoid condensation, allow the antigen strips to warm to room temperature before opening the sealed packets.

Polyvalent Conjugate, 16 mL | REF | EL1704 | CONJ | Poly |

One vial of affinity-purified and peroxidase-conjugated rabbit anti-human antibody (IgG, IgM, IgA). Contains protein, buffer, and preservatives.

Positive Control, 0.3 mL | REF | EL1711 | CONTROL | + |

One vial of human serum. Contains 0.1% sodium azide as a preservative. Requires dilution before use (see Specimen, Controls and Calibrator Preparation, below).

Negative Control, 0.3 mL | REF | EL1712 | CONTROL | — |

One vial of human serum. Contains 0.1% sodium azide as a preservative. Requires dilution before use (see Specimen, Controls and Calibrator Preparation, below).

Cut-Off Calibrator, 0.3 mL | REF | **EL1706** | CONTROL | CAL |

One vial of human serum. Contains 0.1% sodium azide as a preservative. Requires dilution before use (see Specimen, Controls and Calibrator Preparation, below).

Amber Sample Diluent, 100mL | REF | **EL1708** | DIL | SPE |

One vial of PBS containing protein and preservatives.

10X Wash Buffer, 100 mL | REF | **EL0405** | BUF | WASH |

One vial of surfactant in PBS with preservatives. Prepare a 1X wash buffer solution before use.

To prepare a 1X wash buffer solution, mix 100 mL 10X Wash Buffer with 900 mL distilled water and rinse out any crystals. Use only the highest grade purified water for reconstitution of the wash buffer. **It has been observed that some sources of deionized water contain materials that can interfere in the assay.** Swirl until well mixed and all crystals are dissolved.

Substrate Reagent, 16 mL | REF | **EL0009** | SUBS | TMB |

One vial of tetramethylbenzidine (TMB) and hydrogen peroxide in buffer. The Substrate Reagent may turn slightly blue while stored cold, however, the color should return to slightly amber after warming to room temperature. A dark blue color indicates contamination with peroxidase; and, if this occurs, use a fresh bottle.

Stop Reagent, 16 mL | REF | **EL0105** | SOLN | STOP |

One vial 1M sulfuric acid.

Sealing Tape

Two sheets of sealing tape.

MATERIALS REQUIRED, BUT NOT SUPPLIED

1. Distilled water
2. 250 or 500 mL wash bottle *or* automated EIA plate washer
3. 1 L graduated cylinder
4. 12 x 75 mm borosilicate glass test tubes or equivalent
5. 10µL pipettors with disposable tips
6. 100 µL pipettors with disposable tips (100 µL eight-channel pipettors recommended for runs over 48 wells.
7. 1 mL pipette or dispenser
8. Timer
9. Paper towels or absorbant paper
10. Sink
11. Vortex mixer or equivalent
12. ELISA plate spectrophotometer, wavelength = 450 nm

SHELF LIFE AND HANDLING

1. Kits and kit reagents are stable through the end of the month indicated by the label expiration date when stored at 2 to 8°C.
2. Do not use test kit or reagents beyond their expiration dates.
3. Do not expose reagents to strong light during storage or incubation.
4. Allow reagents to warm to room temperature before use.

WARNINGS AND PRECAUTIONS

1. Within the U.S.: For *in vitro* diagnostic use. Outside the U.S.: for research use only.
2. All blood products should be treated as potentially infectious. Source materials from which this product (including controls) was derived have been screened for Hepatitis B surface antigen, Hepatitis C antigen and HIV-1/2 (AIDS) antibody by FDA-approved methods and found to be negative. However, as no known test methods can offer 100% assurance that products derived from human blood will not transmit these or other infectious agents, all controls, serum specimens and equipment coming into contact with these specimens should be considered potentially infectious and decontaminated or disposed of with proper biohazard precautions. CDC and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2. [5]
3. The antigen wells are produced with inactivated histoplasma antigens. After adding patient or control specimens, the strips should be considered potentially infectious and handled accordingly.
4. Sodium azide at a concentration of 0.1% has been added to some reagents as an antibacterial agent. To prevent buildup of explosive metal azides in lead and copper plumbing, those reagents (see Materials Supplied, above) should be discarded into sewerage only if diluted and flushed with large volumes of water. Use copper-free and lead-free drain systems where possible. Occasionally decontaminate the drains with 10% sodium hydroxide (CAUTION: caustic), allow to stand for 10 minutes, then flush with large volumes of water.
5. Do not substitute or mix reagents from different kit lots or from other manufacturers.
6. Use only protocols described in this insert. Incubation times or temperatures other than those specified may give erroneous results.
7. Cross-contamination of patient specimens can cause erroneous results. Add patient specimens and handle strips carefully to avoid mixing of sera from adjoining wells. Decant carefully.
8. Bacterial contamination of serum specimens or reagents can produce erroneous results. Use aseptic techniques to avoid microbial contamination.
9. Perform the assay at room temperature (approximate range 20 to 25°C).
10. Use proper pipetting techniques, maintaining the pipetting pattern throughout the procedure to ensure optimal and reproducible values.
11. The stop reagent contains sulfuric acid. Do not allow to contact skin or eyes. If exposed, flush with copious amounts of water.
12. This test must be performed on serum only. The use of whole blood, plasma or other specimen matrix has not been established.
13. Sodium azide inhibits conjugate activity. Clean pipette tips must be used for the conjugate addition so that sodium azide is not carried over from other reagents.
14. Hyper-lipemic, heat inactivated, hemolyzed, icteric, and contaminated sera must not be tested.

SPECIMEN COLLECTION AND PREPARATION

Serum is the specimen source. No attempt has been made to assess the assay's compatibility with other specimens. Performance characteristics have not been established with hyper-lipemic, heat inactivated, hemolyzed, icteric, or contaminated sera. It is unknown if such specimens will cause erroneous results.

Specimen Collection and Handling

Collect blood samples aseptically using approved venipuncture techniques by qualified personnel. Allow blood samples to clot at room temperature prior to centrifugation. Aseptically transfer serum to a tightly closing sterile container for storage. Separated serum should remain at 22°C for no longer than 8 hours. If the assay will not be completed within 8 hours, refrigerate the sample at 2 to 8°C. If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -20°C or colder. Thaw and mix samples well prior to use.

Specimen, Controls and Calibrator Preparation

Dilute each specimen, control and calibrator 1:101. For example, label tubes and dispense 1 mL of Sample Diluent into each labeled tube. Add 10 µL of specimen, control or calibrator to each appropriate tube containing the 1 mL Sample Diluent and mix well by vortex mixing.

TEST PROCEDURE

Performance characteristics have not been established for procedures that are different from the procedure described below. Different procedures, e.g., different times, volumes, temperatures, or others, may produce invalid results.

1. Bring all reagents to room temperature before use. Remove the Antigen Well packet from cold storage. To avoid condensation, allow micro-well strips to reach room temperature before opening the foil packet. If less than a full plate is to be used, return unused strips to the foil packet with desiccant and reseal completely. Store unused antigen wells at 2 to 8°C. (Note: At the end of the assay, retain the frame for use with the remaining strips.)
2. Fill wells with 1X Wash Buffer solution (see Materials Supplied, above) and allow to soak for 5 minutes. Decant (or aspirate) the antigen wells and tap vigorously to remove Wash Buffer. Blot the emptied Antigen Wells face down on clean paper towels or absorbent paper to remove residual Wash Buffer.
3. Dispense 100 µL of the Sample Diluent into the "blank" wells and 100 µL of each diluted specimen, control or calibrator (see Specimen, Controls, and Calibrator Preparation, above) into the appropriate wells. (Note: For runs with more than 48 wells it is recommended that 250 µL of each diluted sample first be added to a blank microtiter plate in the location corresponding to that in the ELISA wells. The samples can then be efficiently transferred into the Antigen Wells with a 100 µL 8- or 12-channel pipettor.)
4. Cover plates with sealing tape (or place in a humid chamber), and incubate for 60 ± 1 minute at room temperature (20 to 25°C).
5. Remove sealing tape (or remove wells from the humid chamber), and empty the contents of the wells into a sink or a discard basin.
6. Fill each well with a gentle stream of 1X Wash Buffer solution from a wash bottle then empty contents into a sink or a discard basin.
7. Repeat wash (step 6) an additional 2 times.
8. Tap the antigen wells vigorously to remove 1X Wash Buffer. Blot the emptied Antigen Wells face down on clean paper towels or absorbent paper to remove residual 1X Wash Buffer.
9. Dispense 100 µL Conjugate to all wells, using a 100 µL 8- or 12-channel pipettor.
10. Cover plates with sealing tape (or place in a humid chamber) and incubate for 30 ± 1 minute at room temperature (20 to 25°C).
11. Repeat wash steps 5 through 8.
12. Pipet 100 µL of Substrate Reagent to all wells, using a 100 µL 8- or 12-channel pipettor. Begin incubation timing with the addition of Substrate Reagent to the first well. (Note: Never pour the substrate reagent into the same trough as was used for the conjugate.)
13. Incubate for 10 ± 1 minute at room temperature (20 to 25°C).
14. Stop the reaction by adding 100 µL of Stop Reagent to all wells using a 100 µL 8- or 12-channel pipettor. Add the Stop Reagent in the same sequence and at the same pace as the Substrate was added. In antibody-positive wells, color should change from blue to yellow.
15. Gently blot the outside bottom of wells with a paper towel to remove droplets that may interfere with reading by the spectrophotometer. Do not rub with the paper towel as it may scratch the optical surface of the well. (Note: Large bubbles on the surface of the liquid may affect the OD readings.)
16. Measure the absorbance of each well within 1 hour of stopping the assay. Set the microwell spectrophotometer at a wavelength of 450 nm. Zero the instrument on the blank wells, or correct all ODs by manually subtracting the blank ODs.

ELISA Procedure (condensed version)

1. Dilute samples **1:101** in Sample Diluent (e.g., 10 µL + 1000 µL).
2. Soak Wells for **5 minutes** with **1X Wash**, decant.
3. **100 µL** of **sample** for **60 minutes**, decant.
4. Wash 3 times.
5. **100 µL** of **Conjugate** for **30 minutes**, decant.
6. Wash 3 times.
7. **100 µL** of **Substrate Reagent** for **10 minutes**.
8. **100 µL** of **Stop Reagent**, read at λ = 450 nm.

Please see the **PROCEDURE** section for important details.

QUALITY CONTROL

Each plate run (or strips or wells from a single plate) must include the Cut-off Calibrator and the two Controls. If multiple plates are run, include the Cut-off Calibrator and both controls on each plate. It is recommended that until the user becomes familiar with the kit performance, all specimens, controls and the Cut-off Calibrator should be run in duplicate

with the Cut-off Calibrator run twice for a total of four wells. If single wells are used, the Cut-off Calibrator should be run in triplicate. Include a minimum of 1 blank well (containing sample diluent only) for instrument calibration purposes.

The Cut-off Calibrator has been formulated to give the optimum differentiation between negative and positive sera. Although the absorbance value may vary between runs and between laboratories, the mean value for the Cut-off Calibrator wells must be within 0.100 to 0.700 OD units. All replicate Cut-off Calibrator ODs should be within 0.10 absorbance units from the mean value.

Report results as index values relative to the Cut-off Calibrator. To calculate index values, divide specimen and control optical density (OD) values by the mean of the Cut-off Calibrator OD values.

1. The Positive Control index values should be between 2.0 and 4.0.
2. The Negative Control index values should be less than 0.8.

If the Calibrator or controls are not within these parameters, patient test results should be considered invalid and the assay repeated.

The Positive and Negative Controls are intended to monitor for substantial reagent failure. The Positive Control should not be used as an indicator for Cut-off Calibrator precision and only ensures reagent functionality. Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. In the US, regulatory authorities recommend that the user refer to NCCLS C24-A and 42 CFR 493.1256 for guidance on appropriate QC practices.

INTERPRETATION OF TEST RESULTS

To calculate index values, divide specimen optical density (OD) values by the mean of the Cut-off Calibrator absorbance values.

The magnitude of the index value above the Cut-off Calibrator does not indicate the total amount of antibody present.

Cut-off Development.

Focus established the Cut-off to balance both sensitivity and specificity by testing blood donors from the Gulf Coast of the US (n = 151), and histoplasma ID and/or CF positives (n = 95). The Focus ELISA was negative with 93.7% (134/143) of the blood donors, excluding 8 equivocals. The 95 histoplasma ID and/or CF positives were classified into three groups: histoplasma ID positives (n = 51), ID negative and CF ≥32 (n = 30), and ID negative and CF <32 (n = 14). Of the 51 histoplasma ID positives, the Focus ELISA was positive with 86.0% (43/50), excluding one equivocal. Of the 30 histoplasma ID negative and CF ≥32 samples, the Focus ELISA was positive with 59.3%, excluding three equivocals. Of the 14 histoplasma ID negative and CF <32 samples, the Focus ELISA was negative with 83.7% (10/12), excluding two equivocals.

Index	Interpretation
> 1.10	**Antibody Positive**. An index value of > 1.10 indicates antibodies to histoplasma were detected. The presence of antibodies is presumptive evidence that the patient was or is currently infected with (or exposed to) histoplasma.
≤ 1.10 and ≥ 0.90	**Antibody Equivocal**. An index value of ≥ 0.90 and ≤ 1.10 is considered an equivocal result. It is recommended that samples with equivocal results be tested using a different method; or the patient may be re-drawn two or more weeks later and re tested with this assay.
< 0.90	**Antibody Negative**. An index value of <0.90 indicates antibodies to histoplasma were not detected. The absence of antibodies is presumptive evidence that the patient was not infected with histoplasma. However, the sample may have been drawn before antibodies were detectable, or the patient may be immunosuppressed. If infection is suspected, then another sample should be drawn 7-14 days later and tested.

LIMITATIONS

1. The performance of this assay has not been established for screening the general population.
2. The performance of this assay has not been established for ruling out diseases with similar symptoms, e.g., diseases caused by *Mycobacterium tuberculosis, Coccidioides immitis*, *Aspergillus fumigatus*, *Blastomyces dermatitidis*, *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Legionella pneumophila* or other pathogens.
3. The performance of this assay has not been established for matrices other than serum, or visual result determination(s), or monitoring therapy.
4. All results from this and other serologies must be correlated with clinical history, epidemiological data, and other data available to the attending physician in evaluating the patient.
5. The prevalence of infection will affect the assay's predictive value.
6. The test can be positive with persons that are not currently infected with histoplasma. Antibodies may be present because of a past infection.
7. The test can be negative with persons that are currently infected with histoplasma. Samples obtained very early in the infection may not have detectable antibodies.

EXPECTED VALUES

The prevalence of histoplasma antibodies varies depending on age, geographic location, testing method used, and other factors. In the United States, Histoplasmosis is endemic along the Ohio and Mississippi River valleys. Histoplasmosis is not a nationally reportable disease, but is reportable in some states. Where reportable, incidence is about 1 reported case per 100,000 population.

PERFORMANCE CHARACTERISTICS

Relative Sensitivity and Specificity with Samples Submitted for Fungal Testing (n = 618)

Focus and two external investigators assessed relative sensitivity and specificity of the Focus ELISA versus histoplasma complement fixation (CF) using samples submitted for fungal testing, and using Immunodiffusion (ID) as the comparator. Site 1 (n = 299) sequentially selected all samples submitted for aspergillus, blastomyces, coccidioides, and/or histoplasma serology testing (complement fixation and/or immunodiffusion). Site 2 (n = 100) sequentially selected samples that were positive for histoplasma CF and/or histoplasma ID. Site 3 (n = 219) sequentially selected 15 histoplasma confirmed positive samples, nine samples that were presumed positive (CF positive) or confirmed positives (ID positive) for other fungi, and 195 samples submitted for aspergillus, blastomyces, coccidioides, and/or histoplasma serology testing (complement fixation and/or immunodiffusion). Sites 2 and 3 were clinical laboratories located in the mid-west United States. Histoplasma CF included two separate CF tests (mycelial and yeast), and a sample was considered positive if either CF test was positive. Because of the controversial nature of samples with low positive CF results (1:8 and 1:16),[3] Focus analyzed the data based on two separate CF positive ranges: ≥1:8 representing higher sensitivity, and ≥1:32 representing higher specificity. All samples were tested with Histoplasma CF, all fungal CF positives were tested with a corresponding fungal ID assay (e.g., histoplasma CF positives were tested with histoplasma ID), and all Focus ELISA positives were tested with histoplasma ID. Of the 618 samples tested, 74 were histoplasma confirmed positives by ID, 32 were positive for other fungi (one aspergillus confirmed positive, two aspergillus presumed positives, one aspergillus and blastomyces presumed positive, four blastomyces presumed positive, three coccidiodes confirmed positives, and 21 coccidioides presumed positives), and 512 were from patients with an unknown history of infection.

Of the 74 histoplasma confirmed positives, the Focus ELISA was positive with 86.3% (63/73), equivocal with one sample, and negative with ten samples. The histoplasma CF at ≥1:32 was positive with 58.1% (43/74) of the histoplasma confirmed positives, and the histoplasma CF at ≥1:8 was positive with 82.4% (61/74) of the histoplasma confirmed positives.

Of the 512 samples from patients with an unknown history of infection, the Focus ELISA was negative with 83.9% (402/479), positive with 77, and equivocal with 33. The histoplasma CF at ≥1:32 was negative with 93.3% (477/511) of the samples, and the histoplasma CF at ≥1:8 was negative with 88.3% (451/511) of the samples from patients infected with unknown pathogens.

Of the 32 samples positive for other fungi, the Focus ELISA was positive with 20.7% (6/29) samples, equivocal with three samples, and negative with 23. The histoplasma CF at ≥1:32 was positive with 0.0% (0/32) of the samples positive for other fungi, and the histoplasma CF at ≥1:8 was positive with 3.1% (1/32) of the samples positive for other fungi.

Relative Sensitivity and Specificity with Samples Submitted for Fungal Testing (n = 618)

Characteristic	Focus ELISA				Histoplasma CF (Mycelia & Yeast Combined) %	
	%*	Pos	Eqv	Neg	≥1:32	≥1:8
Relative sensitivity with Histoplasma confirmed positives (ID positive)	86.3% (63/73) * 95%CI: 76.2-93.2	63	1	10	58.1% (43/74) 95%CI: 46.1-69.5%	82.4% (61/74) 95%CI: 71.8-90.3%
Relative specificity with unknown pathogens (ID negative when tested by ID)	83.9% (402/479) † 95%CI: 80.6-87.2%	77	33	402	93.3% (477/511) † 95%CI: 90.8-95.4%	88.3% (451/511) † 95%CI: 85.5-91.0%
Cross-reactivity with other fungi (CF and/or ID positive for aspergillus, blastomyces, and/or coccidioides)	20.7% (6/29) ** 95%CI: 10.7-44.9%	6	3	23	0.0% (0/32) 95%CI: 0.0-10.9%	3.1% (1/32) 95%CI: 0.1-16.2%

* Equivocals are excluded from the % calculations.

† One sample was not tested with the Histoplasma CF.

Relative Sensitivity and Specificity versus Histoplasma CF and ID (n = 617)

Focus further evaluated the Samples Submitted for Fungal Testing (described above), by classifying results into four groups based on histoplasma ID and histoplasma CF result. Of the 617 samples (1/618 samples was not tested with CF):
43 were ID positive and CF ≥32, and were used to calculate "Sensitivity";
481 were ID negative and CF <8, and were used to calculate "Specificity".
31 were ID positive and CF <32, and were used to calculate " Reactivity with Intermediate Group 1";
62 were ID negative and CF >8, and were used to calculate "Reactivity with Intermediate Group 2";

One sample was not tested with CF, and it was ID negative and Focus ELISA negative.
The Focus ELISA was 93.0% (40/43) sensitive (histoplasma ID positive and CF ≥32). The Focus ELISA was 88.4% (396/448) specific (histoplasma ID negative and CF<8), excluding 33 Focus equivocals. The Focus ELISA was positive with 76.7% (23/30) of Intermediate Group 1 samples (histoplasma ID positive and CF<8), excluding one Focus equivocal.
Focus ELISA was positive with 52.5% (31/59) Intermediate Group 2 samples (histoplasma ID negative and CF≥8), excluding three Focus equivocals.

Relative Sensitivity and Specificity versus Histoplasma CF and ID (n = 617)†

Characteristic	Histoplasma		n	Focus Reactivity			
	ID	CF		%*	+	Eqv	Neg
Sensitivity	+	≥32	43	93.0% (40/43) 95% CI: 80.9-98.5%	40	0	3
Specificity	Neg	<8	481	88.4% (396/448) 95% CI: 85.4-91.4%	52	33	396
Positivity with Intermediate Group 1	+	<32	31	76.7% (23/30) 95% CI: 57.7-90.1%	23	1	7
Positivity with Intermediate Group 2	Neg	≥8	62	52.5% (31/59) 95% CI:39.1-65.7%	31	3	28

† Excludes one of 618 samples that was not tested with CF.
* Equivocals are excluded from the % calculations.

Cross-reactivity and Interference

In a separate study from above, Focus evaluated cross-reactivity by testing sera that were seropositive for other fungi (n = 29), anti-nuclear antibodies (ANA, n = 17), or rheumatoid factor (RF, n = 12). Focus tested 29 sera that were confirmed positive by ID for aspergillus (n = 10), blastomyces (n = 4), or coccidioides (n = 15). Overall, the Focus ELISA was positive with 25.0% (7/28) of sera that were positive for other fungi. Of the ten aspergillus confirmed positives, the Focus ELISA was positive with 40.0% (4/10). Of the four blastomyces confirmed positives, the Focus ELISA was positive with 50.0% (2/4). Of the 15 coccidioides confirmed positives, the Focus ELISA was positive with 7.1% (1/14), and equivocal with one sample. Of the 17 ANA positives, the Focus ELISA was positive with 5.9% (1/17). Of the 12 RF positives, the Focus ELISA was positive with 8.3% (1/12).

Cross-reactivity and Interference

Population	Focus ELISA			
	% Positive	Pos	Eqv	Neg
ID positive for other fungi **	25.0% (7/28)* 95% CI: 10.7-44.9%	7	1	21
ANA positive	5.9% (1/17) 95% CI: 0.1-28.7%)	1	0	16
RF positive	8.3% (1/12)* 95% CI: 0.2-38.5%	1	1	11

* Excludes one Focus equivocal from the calculation.
** The Focus ELISA was positive with 4/10 aspergillus positives, 2/4 blastomyces positives, and 1/14 coccidioides positives.

Reproducibility

Reproducibility studies included Inter-lot Reproducibility, Inter/Intra-assay Reproducibility, and Inter-laboratory Reproducibility. Focus assessed the device's Inter-lot Reproducibility by testing ten samples on three separate days with three separate lots. Each of the three lots had a different lot of Antigen Wells. Focus assessed the device's Inter/Intra-assay Reproducibility by testing ten samples in triplicate, once a day, for three days, for a total of 90 data points. A clinical laboratory located in the upper mid-west U.S., and a clinical laboratory located in the mid-western U.S., and Focus, assessed the device's Inter-laboratory Reproducibility. Each of the three laboratories tested ten samples in triplicate on three different days.

Reproducibility													
Dx Interpretation	**Sample**	**Intra-assay**		**Inter-Assay by Site**						**Inter-lot**		**Inter-Lab**	
				Site 1		**Site 2**		**Site 3**					
		Index Mean	**CV, %**	**Index Mean**	**CV, %**	**Index Mean**	**CV, %**	**Index Mean**	**CV, %**	**Index Mean**	**CV, %**	**Index Mean**	**CV, %**
Negatives	9	0.13	14.8	0.12	13.3	0.08	1.2	0.18	31.4	0.10	3.8	0.13	38.7
	5	0.34	8.4	0.36	8.6	0.22	11.0	0.45	23.5	0.28	19.4	0.34	32.5
	2	0.37	13.0	0.34	12.9	0.28	29.5	0.49	23.3	0.28	6.3	0.37	29.5
	Mean		12.1		11.6		13.9		26.1		9.8		33.6
Positives	7	1.41	3.5	1.47	10.2	1.32	10.7	1.43	4.0	1.24	7.9	1.41	5.5
	8	2.06	11.3	2.43	4.6	1.99	11.9	1.76	8.3	2.42	11.4	2.06	16.4
	3	3.05	4.2	2.91	12.5	2.96	17.3	3.26	4.6	2.52	6.8	3.05	6.2
	4	3.11	3.3	3.14	8.8	3.03	22.3	3.16	8.5	2.76	10.7	3.11	2.3
	1	3.38	2.6	3.27	14.4	3.45	22.9	3.42	7.2	2.70	8.1	3.38	3.0
	10	3.66	3.7	3.69	10.1	3.64	16.9	3.65	14.2	3.12	8.8	3.66	0.7
	6	5.78	2.7	6.22	10.4	5.65	21.0	5.46	11.0	5.16	8.8	5.78	6.9
	Mean		4.5		10.1		17.6		8.3		8.9		5.9

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